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The Foundation functions chiefly through grants-in-aid in support of research in university medical centers where there are facilities for research in the basic sciences and public health.

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# NUTRITION REVIEWS

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## THE FOOD AND DRUG ADMINISTRATION FACES NEW RESPONSIBILITIES

Nearly 100 years ago a young Oxford mathematics instructor named Charles Lutwidge Dodgson published a children's classic under the pseudonym Lewis Carroll. Its publication resulted from a fantastic tale he told one afternoon to entertain Alice Liddell, one of the young daughters of the dean of the college (*Alice's Adventures in Wonderland* (1865)). In this fantasy Alice experienced many exciting adventures. But if a mid-nineteenth century Alice were to visit a modern American supermarket she would be just as amazed at the wonderful things she would see as she was in her trip down that imaginary rabbit hole in 1865. For she would indeed be in a wonderland of strange, beautiful, delicious, and attractively packaged foods from all parts of the world. She would find fresh, canned, frozen, dried, bottled, and plastic-wrapped foods unheard of in her day. She would also see pre-mixed, precooked, and ready-baked delicacies almost beyond belief.

But the chemical industry and modern food technology have made the job of the food and nutrition chemist also a strange wonderland—a wonderland of flavors, colors, emulsifiers, pesticide residues, conditioning agents, enzymes, gas propellants, antioxidants, vitamins, amino acids, and preservatives. Faced with the ever changing composition of American foods, a chemist in the laboratories of the Food and Drug Administration can truly say, as the Queen said to Lewis Carroll's Alice: "Now here, you see, it takes all the running you can do to stay in the same place" (*Through the Looking-Glass* (1870)).

*Research in Nutrition and Foods.* Research has not been one of the major activities of the Food and Drug Administration. This is not through lack of interest, but because the heavy demands of routine analyses for

enforcement purposes have required most of the limited laboratory space, personnel time, and money. Nevertheless, the various divisions have made significant contributions to the literature of foods and nutrition. With only limited funds available for research, it would be expected that the type undertaken would be largely in analytical methodology, and such has been the case. The Division of Food has been especially active in developing the collaborative studies of the Association of Official Agricultural Chemists. As a matter of fact, the *Journal of the A.O.A.C.* is an unofficial organ of the Food and Drug Administration.

The late Elmer M. Nelson, who at the time of his unexpected death in late 1958 was Director of the Division of Nutrition, was one of a few outstanding leaders in the scientific work of the FDA and its antecedent organizations. He was recognized for his statesmanship in national and international matters dealing with vitamin assay methods and control. It is most fitting, therefore, that it would be his laboratory which developed a chemical method for vitamin D assay to replace the tedious and inexact rat bio-assay method (J. B. Wilkie, S. W. Jones, and O. L. Kline, *J. Am. Pharm. Assn.* **47**, 385 (1958)).

Although work still remains to be done to gain official approval for this method by the U.S.P. and the A.O.A.C., it should ultimately result in great saving of time in the vitamin D<sub>2</sub> analysis of pharmaceuticals and possibly also of foods. Unfortunately it cannot yet be used for the assay of vitamin D<sub>3</sub>, so the chick must still be used for this assay.

It can be said truly that the passing of Elmer M. Nelson marks the end of an era, an era which identified vitamins with rats.

*Special Dietary Foods.* Under the "403

(j)" section of the Federal Food, Drug, and Cosmetic Act, the Food and Drug Administration has authority to promulgate regulations for interstate commerce in foods which are purported to have special dietary uses. This much discussed part of the law reads:

"SEC. 403. A food shall be deemed to be misbranded—

(j) If it purports to be or is represented for special dietary uses, unless its label bears such information concerning its vitamin, mineral, and other dietary properties as the Secretary [of Health, Education, and Welfare] determines to be, and by regulations prescribes as, necessary in order fully to inform purchasers as to its value for such uses."

Consonant with this authority, regulations regarding the labeling of vitamin content and mineral content of foods have been established.

Also under this authority the Administration has established values for the "minimum daily requirements" of man for various of the vitamins. The fact that these are *minimum* requirements and are therefore lower than the Recommended Dietary Allowances of the Food and Nutrition Board of the NAS-NRC (*Publication 589, National Academy of Sciences-National Research Council (1958)*) has caused no small amount of confusion, even among experts.

One of the most controversial regulations promulgated under the 403 (j) section of the Act prescribes the required label declaration for artificially sweetened foods. Such labels must contain the words: "Contains —. . . , a non-nutritive, artificial sweetener which should be used only by persons who must restrict their intake of ordinary sweets." The wording of this declaration was written when saccharin-sweetened foods were designed primarily for diabetics. Since then the significance and extent of obesity as a serious American health hazard has caused widespread manufacture, sale, and use of artificially sweetened foods for

weight reduction or control. Such use of purported "low-calorie" foods has greatly exceeded their use by diabetics, and since weight reduction is often attempted by the do-it-yourself technique, the wording "... who *must* restrict their intake of ordinary sweets" becomes rather unsuitable. Suggestions have been made to modify somewhat this exact wording.

*Enforcement of the Food, Drug, and Cosmetic Laws.* Although most food, drug, and cosmetic manufacturers and producers do their best to supply the American people with safe, pure, wholesome materials at a fair price, there are occasional producers and dealers who are either ignorant, careless, stupid, or downright dishonest. Through long years of patient education and publicity, the Food and Drug Administration has elicited the cooperation of agriculture and industry so that legal actions against violation of the laws and regulations are only rarely necessary. Where misrepresentation, misbranding, short weight, contaminated or decomposed food, or dangerous drugs are found, and where voluntary correction cannot be obtained, the Department does not hesitate to use every legal means at its disposal.

The routine work of the Food and Drug Inspector is usually quite prosaic and unexciting. He inspects production areas, whether farm or factory; examines the premises for poor sanitary practices and sources of rodent or insect contamination; inspects raw materials and production methods; collects samples of foods, drugs, and cosmetics and their ingredients for chemical and microbiological examination; compares the label claims of the packaged materials with the ingredients found in the factory or warehouse; and, where indicated, suggests corrective action to alleviate poor production or manufacturing practices. The samples he collects are sent either to a district laboratory or to the headquarters laboratories in Washington for examination and analysis.

The laboratories of the Administration examine the collected specimens for decomposition, insect and rodent filth; analyze the foods for pesticide residues, vitamins, minerals, and other incidental or intentional additives; and compare the analytical data with the label claims and with the regulatory requirements. Thousands of such samples are analyzed each year on both domestic and imported foods, drugs, and cosmetics and the raw commodities going into their manufacture. The fact that very few of these sample examinations lead to legal action attests not only to the honesty and integrity of the great majority of the producers, but also to the effectiveness of the scientific and enforcement activities of the Food and Drug Administration.

Occasionally outright dishonesty or racketeering is uncovered. One recent case has all the exciting characteristics of a pulp magazine novel. It has been known for a number of years that incubator-reject rotten eggs were finding their way into foods, but evidence which could lead to a conviction was difficult to obtain. Anyone can tell a rotten egg from a fresh egg by taste and smell, but an odor cannot be given a quantitative value and is not readily captured to be presented later to a judge or jury. Also, it is possible to deodorize rotten eggs or to disguise their odor by mixing them with fresh eggs in fluid egg containers. Pains-taking chemical research charted the changes which occur when eggs decompose and those data were found reliable in giving objective evidence of decomposition. In large hatcheries it is common practice to "candle" the incubating eggs at the twentieth day, and those which are not viable are removed and discarded. There is little legal outlet for such rejects, so some unscrupulous people have at times collected, reconditioned, and sold them to food manufacturers and bakers as edible broken, fluid eggs.

In a recent case, evidence was obtained that rotten eggs from certain hatcheries

were being diverted to illegal food use. By the device of an invisible ultraviolet-fluorescent dye, certain cases of the reject eggs were marked for later identification. When the eggs were picked up in the middle of the night by a racketeer's truck, the FDA inspectors with the assistance of local agents followed the truck at high speed through several states. The chase took several days and nights. Finally, the drivers, suspecting that they were being followed, abandoned the truck. But the inspectors continued their surveillance and finally caught the drivers, and, through them, the gangsters responsible. The chain of evidence brought a conviction and so, temporarily at least, this foul practice has been stopped.

The regulatory activities of the FDA include not only food, drug, and cosmetic inspection and control, but also the certification of certain food and drug materials, including certifiable coal-tar dyes, antibiotics, and insulin preparations.

*Food Standards.* Federal laws authorize the FDA to establish standards for foods, and such standards have been promulgated for a large number of foods. These specify the type and amount of required ingredients, list certain optional ingredients which may be used, and regulate the labeling permitted. For some types of foods, standards of fill of container have been issued to prevent the packer from using a deceptively large container or substituting water or sugar solution for the food product. Standards of quality have also been published for some fruits and other foods, so that substandard materials cannot be represented as that of first quality. Substandard food can be sold with appropriate labeling but the alert purchaser will know what he is getting.

Most of the standards are referred to as Definition and Standard of Identity. Adherence to these standards assures the consumer that he will receive a nutritious, safe product, informatively labeled. Unfortunately, the standards are worded in a

legal-scientific jargon which is difficult for the average person to understand, and information about these standards has not been made readily available to the average consumer. Much of the value of the food standards is thus lost because of public ignorance of their significance.

Foods which are not standardized are required to be labeled with a list of ingredients, identified by common or usual names, and, although the percentage composition of the product is not required, it is mandatory that the ingredients be listed on the label in descending order of importance, percentage-wise. This aspect of labeling requirement is probably not as widely known as would be desirable for consumer protection and information.

*Nutrition Education.* One continuous responsibility of the FDA is the campaign against food faddism and nutritional quackery. The fraudulent purveyor of aloe leaves, papaya, seaweed, dried alfalfa, pega palo, or royal jelly—who through ignorance or cupidity makes fantastic claims for his product—can often be dealt with through legal means. The American Medical Association and the Better Business Bureaus are staunch allies of the Government in these cases. But the education of the general public in sound nutrition practice is a matter which demands the full cooperation of every agency of education and government. Members of the staff of this agency, from the Commissioner himself down to the field office inspector, are constantly teaching the principles of good nutrition, both in public addresses and private conversation.

The dramatic successes of nutrition science in eradicating certain formerly widespread deficiency diseases in America have convinced a large segment of the population that *any* human ailment can be cured if one could only find the right vitamin, mineral, protein, amino acid, or combination of them. This is of course nonsense, but the impression prevails. It is therefore not surprising that many unsophisticated people

fall prey to the self-styled nutrition expert who is willing to claim anything for his product, whether it be a vitamin pill or dried alfalfa.

*The Safety of Food Additives.* The term "food additive" may include flavors, pesticide residues, artificially induced radioactivity, antioxidants, emulsifying agents, packaging materials, enzymes, conditioners, microbial inhibitors, preservatives, and many other substances. The amendment to the food and drug laws passed by Congress in 1958—commonly referred to as the Food Additive Amendment—was designed to, and does, place upon the *manufacturer* the legal responsibility for proving the safety of a food additive. Under the previous law, an additive could be banned as harmful only if the Food and Drug Administration obtained proof of harmfulness. With hundreds of chemicals and mixtures of unknown composition being added to foods, it was patently impossible for the FDA scientists to screen more than a few of these additives for safety, with the niggardly funds made available by Congress for this purpose.

This shift in the responsibility for proof of safety of a food additive from the Government to the manufacturer would seem, superficially, to relieve the Food and Drug Administration scientists of a major load of work. In actual fact, however, it adds a stupendous burden because it makes it all the more essential that the FDA have a highly competent corps of specialists in biochemistry, biophysics, physiology, pharmacology, toxicology, and pathology. The toxicological examination of a substance is not a straightforward procedure; there are no simple guidelines—every potentially toxic material is a new problem. And evaluating the data submitted by a manufacturer in support of the safety of an additive is not a matter which can be turned over to a clerk or a computer.

Only experts in the various physical, chemical, biological and medical sciences

have such competence. To have attained the judgment required, these scientists must themselves have been engaged in fundamental research in their respective disciplines, and to maintain this competence they must have the time, the resources, and the freedom to be continuously engaged in basic research. Unfortunately, Congress has not yet seen the necessity of making funds available for this essential aspect of the enforcement of the Food Additive Amendment.

The Federal Government spends less than seven cents per person each year for protection of foods, drugs, and cosmetics against adulteration, misbranding, filth and decomposition; protection against dangerous drugs and cosmetics and the hazards of pesticide residues and radioactivity; and all the other enforcement and research activities of the Food and Drug Administration. This

amounts to less than the cost of one candy bar per person per year. Expressed in another way, Congress appropriates to the Food and Drug Administration each year less than one-sixth of the cost of one military jet bomber.

It is my earnest hope that the Food and Drug Administration will immediately enter into a new, aggressive phase of development in which the essential basic research needed in all aspects of food and nutrition will become a major enterprise. This will require a fundamental change in viewpoint within the organization, and the active consent of Congress as expressed by adequate appropriations.

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## VITAMIN A TOLERANCE TEST AND DISORDERS OF FAT METABOLISM

*Vitamin A tolerance tests were applied to 54 anginal patients. High values for vitamin A paralleled fats but not cholesterol. Coronary atherosclerosis occurred in several types of lipid disorders.*

Since coronary atherosclerosis accounts for so many deaths in our society, it is fitting that investigators are seeking avidly for a means of detecting some metabolic defect which, in susceptible persons, may provide means for prophylactic management. Some measure of success has been achieved in the statistical relationships between coronary disease and increased blood levels of cholesterol and beta-lipoprotein. Further information has come from the use of test meals to measure absorption and utilization of lipids. Total lipids, chylomicrons, unesterified fatty acids, glycerol, triolein and radioactive fats have been studied in this manner, but none is a perfect test.

Vitamin A was employed by J. L. Beaumont and J. Lenègre (*Am. Heart J.* 58,

163 (1959)) to study the tolerance of 54 anginal patients to fat. The authors reported that vitamin A esters behave as a tracer of neutral fat during the first stages of its metabolism.

A neutral vitamin A in oil was given to the subjects as follows: 100,000 units at 9:00 a.m. on the first and second days, none on the third day, and 500,000 units on the fourth. Breakfast was fed immediately after the test dose. Blood was collected on the first and fourth days before the test dose and at three, six, nine, 12 and 24 hours thereafter. The normal ranges for the vitamin A tolerance test were established in a group of 19 patients with cardiac disease but without atherosclerosis.

The 54 patients were divided into the six following groups: seven with primary

hypercholesterolemia, eight with essential hyperlipemia, two with diabetes mellitus and hyperlipemia, one with myxedema, 24 with atypical and usually moderate hyperlipemia, and 12 with normal fasting blood lipids.

The vitamin A tolerance test gave normal values for patients with essential hypercholesterolemia, and normal to elevated values for those with normal fasting blood lipids. However, the test gave abnormally high values for the patients with essential hyperlipemia, and variable results for those subjects with atypical hyperlipemia.

The authors interpreted these data as indicating a group of different metabolic

disorders which have in common a disturbance of lipid metabolism and coronary artery disease. The vitamin A tolerance test seemed to be a means of identifying those subjects who had a disturbance of the preliminary stages of lipid metabolism (absorption, transportation and storage). There was a notable lack of correlation with the serum cholesterol.

The vitamin A tolerance test would appear to be a useful device for further investigation of lipid metabolism, especially in groups of patients who have alterations in rates of absorption, transportation and storage of neutral fats.

### THE NORRBOTTEN STUDY

*The relationship between breast and bottle feeding on growth, ossification, antibody responses and serum electrolytes has been studied in a group of 402 infants in northern Sweden.*

There are many serologic and biochemical differences between human milk and cow's milk. The way in which these differences influence the rapid growth of small infants has become of increasing interest in the last few years, although formerly the differences in chemical composition were thought to have little effect. Therefore, in order to reassess the clinical, chemical and immunologic differences between these two types of milk, a large field study was organized in Norrbotten County in the north of Sweden by O. Mellander, B. Vahlquist, T. Mellbin and collaborators (*Acta Paediat.* **48**, Suppl. 116 (1959)). Their specific aim was to evaluate the influence of these two types of feeding on infection and skeletal development.

The County of Norrbotten lies at the Arctic Circle and the subjects studied were from a county district and a mining town. There were no measurable differences between the two groups and the results were combined. All the children in the study, as well as their mothers during pregnancy, were under strict health super-

vision. Between June 1, 1953, and December 31, 1954, there were 660 births in the two areas studied. Four-hundred and two of these infants completed the total program and are the basis of this report.

The general health and dietary habits of the population were satisfactory and no quantitative or qualitative evidence of malnutrition was known to occur. The diets of all the expectant mothers were fortified with animal protein, iron and vitamins and an attempt was made to provide optimum conditions for the development of the fetus. The infants were seen by the public health nurse every two to four weeks during most of the study and had medical examinations every six to twelve weeks. Blood samples for serological and biochemical analysis were obtained at birth, at seven and a half months of age, and again at 30 months of age. The immunization program consisted of tetanus, diphtheria and pertussis at three, four and a half, and six months of age and influenza vaccine at seven months. Skeletal x-ray examinations were made at seven and a half and 30



months. In addition a dental examination was carried out at 30 months of age.

The incidence of full breast feeding for these groups fell from 80 per cent at birth to 32 per cent at six months of age. Supplemental feeding or wholly artificial feeding consisted of a mixture of half cow's milk and half water with 5 per cent sugar and 1 per cent wheat flour. This mixture gave 550 calories per liter and contained 1.7 per cent protein, 1.5 per cent fat, 8 per cent carbohydrate and 0.4 per cent ash. The intake was limited to 600 to 700 g. at one month, increasing to 800 to 1000 g. at three months. Crushed rusks were added at four months, combined with fresh cow's milk for one group and a commercial dried milk preparation for another. From the age of one week the diet was supplemented daily with 2500 units of vitamin A and 1000 units of vitamin D, and this dose was doubled at three months at which time 25 mg. of ascorbic acid were added. Strained baby foods were introduced at four months and the mothers were given a free hand in feeding after the seven and a half-month examination.

The infants were divided into four groups according to the duration of breast feeding. Group one was at breast for less than two weeks, group two from one to two and a half months, group three from three to six months, and group four for six and a half months or longer. Special attention was given to group one with little or no breast feeding and group four which had been exclusively breast fed for over six months. Systematic analysis of the family size, age of mothers, economic status, general health, nutrient intake during pregnancy, birth weight, season of birth and sex of the four groups failed to reveal any statistically significant differences.

Clinical examination, however, revealed a number of differences between groups one and four. The weight gain at seven and a half months was greater for the artificially fed infants than for the breast fed infants, the gain on the cow's milk formula being

5.52 kg. while on the breast milk it was 4.90 kg. This difference was statistically significant at a probability of .001 or less and at 30 months was somewhat smaller ( $P = .05$  to  $.01$ ). There was also a small but significant difference in height between groups one and four at seven and a half months, the bottle-fed infants being taller, but this disparity disappeared at 30 months of age. In the bottle-fed female infants at seven and a half months there were more ossification centers than in their breast-fed counterparts, but, again, this disparity disappeared at 30 months. The only significant differences for hemoglobin and sedimentation rates were at three months of age when there was a slightly lower hemoglobin and slightly higher sedimentation rate in the bottle-fed infants.

Clinical observation and serologic studies revealed no differences in the incidence of fever or the common communicable diseases. However, the detailed clinical records kept on the incidence of rhinitis, cough, otitis media, upper respiratory infection with fever, and acute diarrhea showed that these five infections were more frequent in the country district. There were a few significant differences between feeding groups, the incidence of these infections being slightly higher for the bottle-fed infants. However, the probability of statistical significance never exceeded the 5 per cent level. When the infants of group one were compared with those for group four for all the infections and for all age groups of the first year, those weaned from the breast during the first two weeks of life had significantly more infections than those of the exclusively breast-fed group with a probability of .01 or less. The authors carefully point out that these data apply only to the incidence of acute infections and that they were unable to determine either the severity or the duration of these infections.

The immunologic studies consisted of antistreptolysin and antistaphylolysin titers measured at seven and a half and 30 months



of age, but no significant differences between feeding groups were found.

The response to immunization was studied by measuring antibody response to diphtheria, pertussis and influenza B antigens. Again no correlation with feeding groups was found.

At seven and a half and 30 months of age biochemical assessment was made of total serum proteins, electrophoretic pattern, calcium, phosphorus, alkaline phosphatase, and sodium and potassium in serum. The gamma globulin levels in groups one, two and three were significantly higher than in group four, and it should be noted that the higher gamma globulin levels were in those groups having a slightly higher incidence of infection but which had received cow's milk, whereas group four had received much less cow's milk during the first six months of life. There were no other differences of importance in the electrophoretic pattern.

The serum calcium concentrations in the exclusively breast-fed group four were significantly higher than in group one, indicating that the serum calcium does not have a direct relationship with the calcium intake. On the other hand, those receiving cow's milk (groups one, two and three) showed significantly higher levels of serum phosphorus than did group four, correlating well with the intake. An alternative explanation would be that these differences reflected a constant calcium-phosphorus product, since the alkaline phosphatase values were significantly higher in the infants receiving cow's milk. The similarity to the biochemical changes in rickets was only superficial since the serum phosphorus levels were also raised and x-ray examination of the bones revealed no sign of rickets in any child. No significant differences between the feeding groups were found respecting any of the other components studied.

Dental examination consisted of noting the time of eruption of the deciduous teeth and the incidence of caries at 30 months.

The type of feeding had no influence on the time of eruption of the deciduous teeth, and there were no clear-cut differences in the overall incidence of caries among the feeding groups.

#### *Summary:*

In general the differences that were found seem clear-cut and to be solely due to the differences in feeding between the various groups, but, as might be expected, most of the differences were statistically significant only when comparisons of the extreme groups were made. Moreover, some of the differences, such as those attributed to growth and ossification centers, existed only for a limited period during early infancy. However, the data concerning the incidence of acute infections of the upper respiratory passages and the gastrointestinal tract are particularly pertinent since they tend to confirm previous observations made under poor conditions of hygiene. Small changes in the gamma globulin concentration were found, but differences in response to immunizations with the usual types of vaccines did not appear to be influenced by nutritional factors. The differences in serum calcium, phosphorus and alkaline phosphatase were noted only at seven and a half months, disappearing at 30 months, and could probably be attributed to the higher phosphorus intake since there was no evidence of rickets in any of these infants who received large doses of vitamin D. It is pointed out that the differences between bottle-fed and breast-fed infants in this particular study have meaning only for the specific cow's milk formula that was used, and cannot be applied to other types of cow's milk feeding.

This well-designed and carefully executed study will become a current classic in studies on infant feeding, and sets a very high standard for the design and execution of further studies on other types of cow's milk formulas.

## CORONARY HEART DISEASE AND DIETARY HABITS

*Statistics of coronary artery disease for 20 countries have been re-evaluated and a distinct association with high intakes of saturated fats and animal proteins has been found.*

The dietary habits of our ancestors as well as ourselves have been given strong emotional and social importance. Foods of animal origin have signified strength, health and vigor, as well as security and affluence. Yet in reading through a sequence of 29 articles in *Nutrition Reviews* on the subject of coronary heart disease in relation to dietary habits, one gains the impression that coronary artery disease and atherosclerosis are more common in men or animals fed large quantities of fats, particularly those of animal origin (*Nutrition Reviews* 6, 176, 293 (1948); 7, 155 (1949); 8, 41, 74, 176, 203, 304 (1950); 11, 72, 191 (1953); 12, 25, 270, 325 (1954); 13, 20, 138 (1955); 14, 3, 95, (1956); 15, 17, 353 (1957); 16, 1, 102, 129, 263 (1958); 17, 55, 62, 102, 147, 159 (1959).

What one does not find, however, is a statement of the progressively longer survival or better state of health of those same people who may endanger their health thrice daily at the dinner table. Are we magnifying a scientific measurement out of its true proportion? Dr. George V. Mann stated in his address at the recent A.M.A. Convention, "The evidence will not justify very low fat diets, very low cholesterol diets, the addition of lipotropic agents, the use of supplements of vitamin B<sub>6</sub>, or the addition to diets of the so-called essential fatty acids" (*Prog. 108th Annual Meeting of the A.M.A.*, p. 87, (1959)).

N. Jolliffe and M. Archer (*J. Chron. Dis.* 9, 636 (1959)) have evaluated data based upon records of the World Health Organization and the Food and Agriculture Organization. These data were collected from 20 countries and furnished comparisons between death rates from coronary heart

disease and dietary as well as environmental factors.

The authors stressed that one must consider several factors in interpreting such information. First to be taken into account are the relative efficiencies of the individual data-gathering systems. Also, the amount of food available is a residual figure and the final value is therefore subject to the variables of production, import, export, animal consumption, non-food use and waste. And finally, the dietary data are for food available at the retail level and, therefore, do not exactly represent consumption figures. There could well be greater emphasis upon one of the most important uncertainties in the kind of data gathered, namely, the effect on fat utilization of an excessive intake of calories from any sources beyond actual need.

Next they reviewed the interpretations of others. A. Keys in his report (*J. Mt. Sinai Hosp.* 20, 118 (1953)) relied upon data from six countries, and showed a relationship between the proportion of total fat calories available in the national diet and the death rates from degenerative heart disease for men in selected age groups. J. Yerushalmy and H. E. Hilleboe considered all the countries for which adequate data were available, but did not consider the quality of the fats eaten (*N.Y. J. Med.* 57, 2343 (1957)).

The National Dairy Council (*Dairy Council Digest* 28, 1 (1956)) stated that "no relationship is apparent between incidence of arteriosclerotic and coronary heart disease and any element of diet listed." (The Council listed total calories, total protein, animal protein, total fat, animal fat, vegetable fat, per cent calories total

fat, per cent calories animal fat and per cent calories vegetable fat.)

The point stressed by Jolliffe and Archer is that none of these investigators considered the quality of fat (*i.e.* saturated *vs.* polyunsaturated) in their statistical evaluation. They felt that the relationship between coronary heart disease and the intake of saturated fats as implied by B. Bronte-Stewart (*Lancet* I, 521 (1956); *Brit. Med. Bull.* 14, 248 (1958)), L. W. Kinsell (*Fed. Proc.* 14, 661 (1955)) and H. M. Sinclair (*Lancet* I, 381 (1956)) must pass a rough test by showing general agreement with such data. Accordingly, these authors employed the United Nations data in order to indicate additional lines of investigation and to test the hypothesis that "quality of fat" is a factor in the incidence of coronary artery disease.

The countries studied included those evaluated by Yerushalmy and Hilleboe. The authors posed the following problem: can death rates from "degenerative heart disease" (including arteriosclerotic heart disease, chronic endocarditis, and other degeneration of the heart) for men aged 55 to 59 years from 20 countries, be related statistically to dietary data or to other factors?

They also evaluated "combined categories" of heart disease (vascular lesions affecting the central nervous system, chronic rheumatic heart disease, arteriosclerotic and degenerative heart disease, other diseases of the heart, and hypertension with mention of heart disease), and "arteriosclerotic heart disease" alone. These categories were compared statistically with total death rates, average total available calories, total fat intake, saturated fat intake, unsaturated fat intake, animal protein intake, and with a social factor, *i.e.*, the number of telephones per 100 persons.

Categorically, the authors considered fats to be saturated (eggs, chicken, sheep, beef, pig, cow's milk, cocoa bean, coconut, lard, tallow); monounsaturated (olive oil);

or polyunsaturated (peanut, linseed, pecan, walnut, safflower, corn, cottonseed, sunflower, sesame seed, barley, wheat, rapeseed, marine mammals and teleosts). They compared each category of cardiac disease with each environmental factor. Furthermore, they considered more than one factor at a time to determine co-variance.

The results showed that the death rate from degenerative heart disease was related statistically to per cent of saturated fat (0.8259), per cent of animal protein (0.8016), per cent of fat as total calories (0.7321) and number of telephones per 100 persons (0.6901). A negative correlation existed when per cent of calories as unsaturated fat was considered ( $-0.3218$ ).

Multiple correlations made it evident that some of the factors depended upon each other, *e.g.*, total per cent of fats as calories and total calories supplied by unsaturated fats. Actually little more of the variance was accounted for by considering more than one factor at a time. By means of beta coefficients they were able to compare the relative effects of each variable on a regression line and to determine the relative contribution of each factor to the correlation coefficient.

Thus they ascertained that the intake of saturated fats was the most important factor and the next most important was intake of animal protein.

They postulated that as the economic wealth and caloric intake of a country increase, this increase consists mostly of fat, particularly saturated fats which accompany animal proteins. The negative correlation found for polyunsaturated fats confirms this theory for, as animal foods become abundant, vegetable foods may be used less.

A significant correlation was found between the number of telephones and degenerative cardiac disease. The number of telephones also correlated with the consumption of saturated fats.

While specific information about arteriosclerotic heart disease alone was available

for only 14 of the 20 countries, and hence the significance of the data weakened, yet the correlation with consumption of saturated fats was so high (0.9136) that one can scarcely discredit its validity. The correlation with animal protein was second (0.8333) and paralleled that for the mixed category.

The authors recognized that some people believe the increase in coronary artery disease is an artifact produced by better diagnostic and recording systems, older age of patients and greater public awareness. Nevertheless, they considered it likely that a tenfold increase in incidence from one country to another was significant. Furthermore, the present investigation intended to determine why the present rate is so high, and not why or whether it has been increasing. Finally, the authors pointed out that the correlations here demonstrated are in accord with the results of laboratory investigations and hence are credible.

Unfortunately there is no adequate appraisal of what may be a very important relationship, namely, an unfavorable effect

from habitually consuming more calories than are needed by a large proportion of the population. There are indications that chronic consumption of calories above expenditure requirements may be an important factor in its effect upon cholesterol regulation and atherosclerosis. A trend in this direction might well be correlated with the same positive factors recorded by the authors, including a high intake of total fats, animal fats, animal proteins, saturated fats and such socio-economic factors as the number of telephones.

Nevertheless, this study is a significant contribution to a field of investigation fraught with opinion, confusion and doubt. The authors have demonstrated a distinct correlation between arteriosclerotic heart disease and the eating of saturated fats and animal protein. Yet they do not claim that this is the only factor, nor do they suggest sweeping revisions of our nutritional pattern. Cool deliberation and critical investigation will in time provide a basis for intelligent advice.

### HUMAN LIVER GLYCOGEN LEVELS

*The glycogen content of liver biopsies from 37 individuals was determined and no relationship was noted between the glycogen level and the nutritional status as judged by body weight.*

The level of liver glycogen, the major carbohydrate store of the body, depends upon the immediate nutritional history of the individual, providing there is no abnormality in the structure of the glycogen or in glycogen production. In various mammals the amount of glycogen in the liver varies between 2 and 8 per cent when based upon wet weight of the organ. Liver glycogen is a very labile substance and in laboratory animals a fast of 24 hours or longer will almost completely deplete the liver of its glycogen. While numerous values are given in the literature for the glycogen content of the liver of most experimental animals, information on the glycogen content of the

livers of normal humans is not nearly as extensive.

Recently, however, J. E. Sokal and K. E. Gerszi (*J. Lab. Clin. Med.* **53**, 876 (1959)) have reported on the glycogen contents of the livers of 37 individuals. In all cases the biopsies of liver were obtained during the course of either a diagnostic laparotomy or a surgical procedure, with great care taken to obtain normal biopsies, particularly in those cases where there was malignancy present. It should be noted that, while the preoperative nutrition and anesthesia (cyclopropane often supplemented by the muscle relaxor succinylcholine) were similar, all the patients had been fasting 14 hours prior to surgery.

This would automatically reduce the glycogen content of the liver.

During the preoperative period, the patients were given oral diets offering between 1400 and 1900 calories per day with 125 to 200 g. of carbohydrates. However, they did not always eat all that was given them.

Of the 37 patients studied, 31 had no evidence of jaundice and seven were jaundiced. In the nonjaundiced patients, it was found that the glycogen content of the liver expressed as grams of glycogen per 100 g. of liver was  $2.22 \pm 0.27$ , while the glycogen content of the livers of the seven jaundiced patients had a similar mean value of  $2.12 \pm 0.46$ . Between particular patients, however, the variation in the glycogen content of the liver was quite large, ranging from 0.34 to 5.98 g. per cent, but this variation could not be correlated with the patients' weight.

These investigators compared their values to those obtained by D. S. MacIntyre, S. Pedersen and W. G. Maddock (*Surgery* **10**, 716 (1941)), N. F. Young, J. D. Abels and F. Homburger (*J. Clin. Invest.* **27**, 760 (1948)) and J. A. Hildes, S. Sherlock and V. Walshe (*Clin. Sci.* **7**, 287 (1949)). Analysis of variance failed to show heterogeneity among the four groups. MacIntyre and associates and Young and co-workers obtained liver samples during biopsy while surgical procedures were being carried out on anesthetized patients, while Hildes and associates obtained the specimens by needle

biopsy from unanesthetized\* individuals. It should be noted that all investigators subjected the patients to at least an overnight fast before obtaining the biopsies. From the combined series of data it appears that under these conditions of moderate fasting the upper limit of liver glycogen is about 6 per cent.

The fact that glycogen has a very rapid turnover rate (so that the concentration of liver glycogen would be primarily determined by the nutritional events of the 24 to 72 hours preceding the biopsy) may explain why no relationship was noted between liver glycogen and the apparent nutritional status of the patients.

Although it has been reported that levels of glycogen in individuals with glycogen storage disease can go as high as 12 to 16 per cent, most of the values are below such a level and there may be considerable overlapping between the glycogen levels in this disease and individuals with normal liver glycogen. Both MacIntyre and associates and Young and co-workers reported that patients who were given large glucose feedings before obtaining the liver biopsy had glycogen levels ranging from 3.2 to 9.7 per cent.

In summary, these investigators have added quantitative data as to the glycogen content of the liver of normal patients who had been fasted overnight. While the glycogen content of nonfasted individuals would undoubtedly be higher, at present such data are not available.

## NUTRITIVE VALUE OF PLANTS AS AFFECTED BY GEOGRAPHIC LOCATION

*The environment of a plant may influence its nutritional value. Turnip greens from Blairsville, Georgia, possess vitamin B<sub>12</sub> activity, absent in the same crop in Experiment, Georgia.*

Both the atmosphere (or top environment) and the soil (or root environment) can influence the composition of plants (*Nutrition Reviews* **9**, 189 (1951); **12**, 310, (1954); **13**, 337 (1955)). The importance to

animal nutrition of such environmental influences has not received much attention, with the exception of well-known mineral deficiencies (phosphorus, cobalt, copper) or toxicities (molybdenum, sele-

nium) which have been noted in certain soil types and in certain areas of the world.

The reports of differences in animal growth response to turnip greens grown at several locations is, therefore, of interest (L. F. Gray, M. Speirs, and G. Matrone, *J. Nutrition* 63, 345 (1957)).

Corn, cowpeas, and turnip greens (crops widely used in human diets in many areas of the South) were produced from a common seed stock at three locations: Blairsville and Experiment, Georgia, and Raleigh, North Carolina. The corn and cowpeas were harvested at maturity and the turnip greens were harvested when full-grown but without blooms. The corn and cowpeas were stored in the harvested dry form. The turnip greens were dried at 55° to 60° C in a force draft oven, and were then deribbed and stored in air-tight containers.

The experiments were designed to study the influence of location on the nutritive quality of each of the three crops. To evaluate nutritive quality, a basal diet was fed weanling rats consisting of 40 per cent ground corn, 30 per cent ground cowpeas, 10 per cent ground turnip greens, 5 per cent fat, 1 per cent salt, and 14 per cent sucrose. Viosterol was given to each rat weekly by dropper. Any diet supplements were added to the basal diet at the expense of an equal weight of sucrose.

The basal diet was nutritionally incomplete since it supported growth in weanling rats only to the extent of about 60 per cent of that obtained on a normal stock diet. There was, however, a highly significant effect of location on the nutritive value of the turnip green component. In two different crop years the turnip greens from Blairsville, Georgia, were responsible for an approximate 20 per cent improvement in body weight gain when compared with the similar crop grown at Raleigh, North Carolina, or at Experiment, Georgia.

Additional studies were conducted in which diet supplements were used to deter-

mine the factors responsible for growth stimulation in the Blairsville crop.

Egg albumin, and methionine or vitamin B<sub>12</sub> improved the diets containing turnip greens from either Blairsville or Experiment. Furthermore, these supplements eliminated the previously observed differences between the greens grown at the two locations. On the other hand, a vitamin mixture containing all of the B vitamins, except vitamin B<sub>12</sub>, produced only a slight growth stimulation in the diet containing Blairsville turnip greens, but produced a significant response when added to the diet with Experiment turnip greens.

The difference in nutritive value of the turnip greens grown at the two locations was not caused by minerals, since the ash of one, when added to the diet containing the other, did not alter the nutritive value.

The results suggested, rather, that the basal diet employed was deficient in methionine, and that the vitamin B<sub>12</sub> response could be explained by its sparing action on the methionine requirement. Microbiological assay for methionine indicated that the turnip greens grown at Blairsville contained approximately 4.5 mg. per gram (air-dry basis) while those grown at Experiment contained 3.4 mg. per gram. Moreover, a similar difference in total crude protein was noted in the same crop at the two locations.

Assay for vitamin B<sub>12</sub> activity, using *Lactobacillus leichmannii* 4797 and *Ochromonas malhamensis* 11532, indicated a striking difference in favor of the turnip greens grown at Blairsville. These averaged about 7 millimicrograms per gram in vitamin B<sub>12</sub> activity, contrasted with an average activity of less than 1 millimicrogram per gram for the crop grown at Experiment.

These results are of considerable interest since there are only two naturally occurring substances known which possess vitamin B<sub>12</sub> activity for the animal and for *Ochromonas*, namely cyanocobalamin and vitamin B<sub>12-III</sub> (M. E. Coates and J. E. Ford, *The*

*Biochemistry of Vitamin B<sub>12</sub>*, p. 36. *Biochem. Society Sym. 13, Cambridge University Press, 1955*). They are of particular interest since most of the evidence to date indicates that vitamin B<sub>12</sub> in any significant amount is not present in the leaves of higher plants.

In a more recent publication, these workers have attempted to characterize more completely the vitamin B<sub>12</sub> activity of turnip greens grown at Blairsville, Georgia (Gray and L. J. Daniel, *J. Nutrition* **67**, 623 (1959)). Crops grown at Blairsville and at Experiment were compared in a basal corn-soybean type diet low in vitamin B<sub>12</sub> which was fed to chicks. The results indicated clearly that Blairsville turnip greens contained vitamin B<sub>12</sub> activity while the crop grown from the same seed stock at Experiment did not. Growth responses to crystalline vitamin B<sub>12</sub> were similar to those obtained by the Blairsville turnip greens. Furthermore, the vitamin B<sub>12</sub> content of the livers of chicks on the Blairsville diet was twice as high as that when the turnip greens from Experiment were fed.

All evidence thus pointed toward the presence of vitamin B<sub>12</sub> in the turnip greens grown at Blairsville, Georgia, and the absence of the vitamin in the same crop grown at Experiment.

The identity of the vitamin B<sub>12</sub>-active substance was investigated using electrophoresis and chromatography. By these criteria, there was no evidence that the substance in the Blairsville turnip greens differed from cyanocobalamin.

The authors recognize that all evidence to date supports the view that original sources of vitamin B<sub>12</sub> are confined to microorganisms. Since the turnip leaves were carefully washed, soil microbe contamination would not seem very likely. However, the possibility that vitamin B<sub>12</sub>-producing bacteria are living epiphytically on the turnip green leaves in the Blairsville location is recognized as a possibility.

These results are of particular interest since they again emphasize that the environment in which a plant is grown can have influences measurable by nutritional studies with animals.

## ESTROGENS IN PASTURE FORAGES

*Many pasture plants common in temperate zones have been shown to contain estrogens, but these levels have not been sufficient to suggest that they might cause changes in milk composition.*

The discovery that estrogens are present in clovers and in some grass varieties at certain times of the year has stimulated the assay of a large number of pasture plants by G. S. Pope, M. J. McNaughton and H. E. H. Jones (*J. Dairy Res.* **26**, 196 (1959)). Their results are of particular interest since there is such a widespread use of estrogens in fattening operations with sheep and cattle, species which normally obtain a large proportion of their feed from pastures.

While all of the plants they assayed were found in British pastures, the widespread use of many of these plants in temperate zones around the world increases the appli-

cation of their findings. Estrogens have become of increasing concern in the nutrition of animals since E. M. Bickoff *et al.* (*Science* **126**, 969 (1957)) demonstrated the presence of coumestrol in *Trifolium repens*, the common ladino clover. They showed that its estrogenic potency was about 30 times greater than that of genistein or biochanin A, compounds which have been found in subterranean clover by R. B. Bradberry and D. E. White (*J. Chem. Soc.* **4**, 3447 (1951)), a plant which under certain conditions, at least, has produced a pathological effect in breeding sheep because of its



excessive estrogen stimulation (H. W. Bennets, *J. Depart. Agric. West. Australia* **21**, 104 (1944)).

Additional concern over the presence of estrogens in pasture plants has arisen since the demonstration by S. J. Folley (*Biochem. J.* **30**, 2262 (1936)) and by Folley, H. M. S. Watson and A. C. Bottomley (*J. Dairy Res.* **12**, 1 (1941)) that estrogens administered to lactating cows can cause an increase in the percentage of fat and non-fatty solids in the milk.

The studies of Pope, McNaughton and

Jones with the immature mouse indicated the presence of estrogens in alfalfa, in various species related to alfalfa, in clovers and in a number of grasses. However, none of the levels was high enough to produce changes such as those described by Folley. Nevertheless, since these observations were not closely related to environmental conditions, while reassuring in themselves, they do not rule out the possibility for circumstances which may result in such high levels of estrogen as those that produced pathological effects in Australia.

### THE LAXATIVE EFFECT OF DIETARY ROUGHAGE

*Feeding wheat bran to pigs increased the water content of their feces. The effect was reproduced with pure cellulose fed in a fibrous form, but not with powdered cellulose.*

The practice of adding wheat bran to a human diet in order to increase bowel movements and make them easier is well known. P. H. Cooper and C. Tyler (*J. Agr. Sci.* **52**, 332, 340 (1959)) have reported a recent study of the mechanism for this effect, using the pig whose digestive system has some similarities to that of man.

In the first experiment two newly weaned pigs were used. For three weeks pig A received two daily feeds of 750 g. dry meal and 1750 ml. water, with the meal made up of 60 parts ground maize, 30 parts ground barley, 10 parts fish meal and 1 part salt. Pig B received a similar treatment with 25 parts of wheat bran and 10 parts of starch introduced into its meal of maize. This change increased the cellulose content (as determined by the procedure of L. E. Wise, M. Murphy and A. A. D'Addieco, *Paper Trade J.* **122**, 35 (1946)) from 4.3 per cent of the dry meal to 9.2 per cent, and the crude fiber content from 2.4 to 4.8 per cent.

In a 24-hour period pig A passed six stools with a total weight of 700 g., while pig B passed ten stools with a weight of 1400 g. Much of this difference in weight consisted of water, while the percentage of dry matter in the two series of stools averaged 29.6 and 19.6 respectively. The 24-hour output of

dry matter was 206 g. for pig A and 277 g. for B, and included 40 and 71 g., respectively, of undigested cellulose.

At the end of the experiment the pigs were killed four hours after their morning feed, and the digestive tract was rapidly tied off into sections. The total dry matter in the tract was very similar for the two pigs, being 640 g. for pig A and 680 g. for pig B. However, there were large differences in the total moisture content, with values of 2130 and 3170 g. respectively. The greatest differences were found in the cecum, colon and rectum, while there was less difference in moisture content in the earlier portions of the tract.

The second experiment was designed to test whether the laxative effect of the bran could be reproduced by adding cellulose alone to the same basal diet. The first preparation was in a finely powdered form containing 80 per cent cellulose and 20 per cent hemicellulose. The amount fed to each pig was gradually increased to 25 per cent for one and 10 per cent for the other. However, this powdered cellulose preparation had no laxative effect, although large weights of dry matter were excreted. The stools were in the form of hard pellets and their mean



dry matter contents were 38 and 43 per cent, respectively.

The diets were then changed. The first pig was transferred to a fibrous cellulose preparation, fed at the 5 per cent level. This consisted of filter paper cake (without asbestos) obtained in block form and macerated with water before being mixed with the dry food. Analysis showed it to be pure cellulose. For the second pig the powdered cellulose was still used but the level was reduced to 5 per cent.

There was a considerable difference in the excretions of the two pigs. The stools of pig C receiving the fibrous cellulose contained 24 per cent of dry matter, while those of pig D receiving the powdered material were considerably drier, containing 34 percent dry matter. Pig C also excreted a greater weight of dry matter so that the 24-hour wet weight of its stools was 1250 g. as compared to only 740 g., for pig D. *In vitro* tests with the two cellulose preparations also demonstrated that the powdered preparation absorbed and retained much less water than the fibrous one.

In further experiments, the fibrous cellulose was added (at the expense of starch) to a purified diet made up of 50 parts dried skim milk, 44 parts starch, 5 parts dried brewers yeast and 1 part salt. Adding 15 and 25 per cent of fibrous cellulose resulted in extremely wet stools with only 17 and 15 per cent, respectively, of dry matter as compared with the values of 26 to 34 per cent when little or no cellulose was added to the same basal diet. This meant

that when the 25 per cent level of cellulose was fed, 1660 g. of water were excreted per 24 hours in the stools, but only 300 g. in the urine.

The authors also report the time pattern of excretion throughout the 24 hours in each experiment. They conclude that, in general, excretion takes place during periods of maximum flow of gut contents from the small into the large intestine, and is not necessarily related to the actual mass of material present in the lower gut. This is in agreement with the conclusions of K. L. Blaxter, N. McC. Graham and F. W. Wainman (*Brit. J. Nutr.* 10, 69 (1956)) who made their observations on sheep.

Cooper and Tyler also found large differences in the dry matter content of the stools produced at different points in the 24-hour cycle. In general, those produced in the early morning (which presumably had been longest in the lower gut) were the driest, as might be expected.

The general conclusion that can be drawn from this work is that it is not the cellulose content per se of wheat bran that gives it its laxative effect. This was shown by the failure of a cellulose preparation in powdered form to reproduce the laxative effect of bran. It may be, however, that the physical condition or structure of the cellulose and other relatively indigestible polysaccharides in bran are responsible for the laxative effect because of their power to retain water. Pure cellulose in fibrous form seemed to have an effect very similar to that of bran and to illustrate this mode of laxative action.

### VITAMIN B<sub>12</sub> INTERRELATIONSHIPS

*Dietary riboflavin and folacin appear to spare vitamin B<sub>12</sub> in weanling rats on a low B<sub>12</sub> diet. Choline may have the opposite effect.*

Dietary vitamin B<sub>12</sub> is stored by mammals primarily in the liver and kidney. R. A. Harte, B. F. Chow and L. Barrows (*J. Nutrition* 49, 669 (1953)) and R. F. Schilling (*Am. J. Clin. Nutrition* 3, 45 (1955)) have

shown that the vitamin can be firmly attached to globulins and seems to be held tenaciously in emergency states. Liver storage is increased when the vitamin is increased in the diet (A. E. Schaefer, W. D.

Salmon and D. R. Strength, *Proc. Soc. Exp. Biol. Med.* **71**, 193 (1949)).

L. N. Ellis, B. J. Duncan and I. B. Snow (*J. Nutrition*, **67**, 185 (1959)) have used the storage of vitamin B<sub>12</sub> in the liver to indicate the interrelationship between vitamin B<sub>12</sub> and riboflavin, folacin and choline at levels likely to occur in human diets. They measured the storage of B<sub>12</sub> in the livers of male weanling rats that had been subjected to a period of low intake of the vitamin supplemented by either low or high amounts of the other three vitamins. The experimental period lasted four weeks. Food intake was equalized between the litter mates and the animals were weighed weekly.

A fivefold dietary increase of riboflavin resulted in a small increase in total vitamin B<sub>12</sub> in both liver and kidney when calculated per gram of dry tissue or nitrogen. This indicates a sparing action of riboflavin at sub-optimal levels of vitamin B<sub>12</sub>. The amount of vitamin B<sub>12</sub> per gram of fresh tissue or per total organ was similarly increased in the liver, but a decrease was observed in the case of the kidney because of hydration and lower kidney weight.

The ratio of free to bound vitamin B<sub>12</sub> in these organs increased on the high-riboflavin diet, the percentage of free vitamin B<sub>12</sub> rising from 88 to 100 per cent in the liver and, insignificantly, from 79 to 81 per cent in the kidney. It is not known whether this change made the vitamins more or less available for metabolism. Apparently the mechanism for storage under these conditions did not involve protein binding. Although the nature of the free vitamin B<sub>12</sub> was not clear from these studies, the vitamin B<sub>12</sub> of human liver has been found to be readily available to *Euglena gracilis* (W. R. Pitney, M. F. Beard and E. J. Van Loon, *J. Biol. Chem.* **212**, 117 (1955)).

The authors point out that, although the increase of stored vitamin B<sub>12</sub> on the high-riboflavin diet could have resulted from an increased release of vitamin B<sub>12</sub>-active fragments from riboflavin during digestion, an actual decrease of stored riboflavin on

this diet suggests a metabolic interrelationship.

A fivefold increase of folic acid in the diet resulted in a small, statistically non-significant, increase of total vitamin B<sub>12</sub> in the liver and kidney, but in this case the increase occurred primarily in the bound form. Furthermore, increasing the folic acid of the diet resulted in significant increases of hemoglobin and of stored folic acid.

In contrast to the results with riboflavin and folic acid, a twofold increase of choline in the diet caused a significant decrease of total and free vitamin B<sub>12</sub> in both liver and kidney to approximately the level found with a very deficient diet. The decreased storage occurred with little or no change in the hemoglobin level or in the storage of choline in the liver and kidney.

The authors are of the opinion that, although the decreased storage of vitamin B<sub>12</sub> may have resulted from renal lesions and fatty infiltration of the organs during the two weeks depletion period on a choline-free diet, the negligible decrease in nitrogen content contradicts such changes as being major factors. Moreover, animals on a low-choline diet were able to deposit vitamin B<sub>12</sub>, when added to the diet, at a level comparable to that found in animals on either the low-riboflavin or low-folic acid diets. The authors conclude that the present studies provide no material for interpretation.

When a comparison of results from each of the three series is made, it is seen that at each dietary level of vitamin B<sub>12</sub> the total vitamin B<sub>12</sub> calculated per gram of body weight was highest for the folic acid diets. The lowest value of vitamin B<sub>12</sub>, on a fresh weight basis, was 0.037 µg. per g. of fresh liver for the diet deficient in both vitamin B<sub>12</sub> and choline. This value is higher than that reported by H. E. Scheid, M. M. Andrews and B. S. Schweigert (*Proc. Soc. Exp. Biol. Med.*) **78**, 558 (1951)) (0.026 µg.), as well as that reported by M. J. Burns and Salmon (*J. Agr. Food Chem.* **4**, 257 (1956)) (0.005 µg.).

## POTASSIUM-LYSINE RELATIONSHIPS

*Increasing the potassium content of a lysine-deficient diet appears to compensate partially for the deficiency.*

It has been demonstrated by R. E. Eckel, C. E. Pope II and J. E. C. Norris (*Arch. Biochem. Biophys.* **52**, 293 (1954)) and M. Iacobellis, E. Muntwyler and C. L. Dodgen (*Am. J. Physiol.* **185**, 275 (1956)) that in the rat a loss in muscle potassium is at least partially compensated by a gain in free lysine, which acts as a cation. This fact suggested to S. N. Gershoff *et al.* (*J. Nutrition* **67**, 29 (1959)) that a converse compensation might occur. The effect of altering potassium levels in diets containing varying quantities of lysine was therefore studied.

Groups of eight male weanling Charles River CD (Caesarian delivery) rats, housed in group cages, were used for this study. The diets were composed primarily (89.5 per cent) of a commercial dry breakfast cereal containing 3.12 per cent nitrogen (mostly from rice and wheat gluten and from small amounts of skim milk). The available lysine was found to be only 0.31 per cent. When this diet was supplemented with lysine, rat growth equal to that obtained with isonitrogenous casein-containing diets was achieved.

Other components of the basic diet included 4.0 per cent corn oil, 1.0 per cent cod liver oil, 0.3 per cent choline and adequate vitamin supplements. Diets two, four and six contained 4 per cent Salts IV, and diets one, three and five contained 1.2 per cent disodium phosphate and 2.8 per cent Salts IV minus its potassium salts.

Analysis for lysine and potassium showed that diet one contained 0.28 per cent lysine and 0.14 per cent potassium. Diet two contained 0.28 per cent lysine and 0.72 per cent potassium. Diet three contained 0.53 per cent lysine and 0.14 per cent potassium. Diet four contained 0.53 per cent lysine and 0.72 per cent potassium. Diet five contained 1.28 per cent lysine and 0.14 per cent

potassium. Diet six contained 1.28 per cent lysine and 0.72 per cent potassium. Sodium content of the odd numbered diets was 1.27 per cent and for the even numbered ones, 0.96 per cent. The diet and water were given ad libitum.

After six weeks, the animals were killed and samples of liver, shaved skin and muscle were obtained for analyses of potassium and sodium, nitrogen and amino acids.

Differences in growth were related to the lysine but not to the potassium content of the diets. During the third week, the rats in group one started losing hair and by the end of the experiment showed marked alopecia. Although some of the rats in group two lost some hair during the six weeks, the least involved rat in group one lost considerably more hair than the most involved animal in group two. This, the authors suggested, was clearly a protective effect of increased potassium in diets deficient in lysine.

Histological changes most suggestive of a protective effect of excess dietary potassium in the presence of a lysine deficiency included the liver, in which the amount of sudanophylic material could be correlated with the dietary lysine levels. The livers of rats from groups one and two had moderate amounts of lipid and those of groups three and four had minimal amounts, while those of the two highest lysine groups showed only the minute traces of lipid seen in livers of normal rats. A potassium effect was apparent only at the 0.28 per cent lysine level, where it was associated with a reduction in the amount of liver lipid.

The thickness of the epiphyseal cartilage of the distal end of the radius was measured with the aid of a calibrated ocular grid. At the lowest lysine level, a distinct thinning of the epiphyseal plate could be seen. At

each level of lysine, it was observed that increasing the potassium level of the diet was accompanied by an increased thickness of the epiphyseal plates. However, not all differences were statistically significant.

Various other histological changes could not be related to dietary potassium levels. At the various lysine levels fed, the potassium content of the diets had no significant effect on the tissue concentrations of nitrogen, sodium or potassium. The lysine content of the diet did not affect the sodium and potassium concentrations of muscle and liver, but markedly increased the sodium and potassium content of the skin. There also appeared to be a slight effect of high lysine diets in increasing the nitrogen concentrations of the tissues examined.

Differences in the amino acid paper chromatographic patterns of the various dietary groups were not marked, but a decrease in dietary lysine was associated with increases in muscle lysine and histidine and in the spot representing liver phenylalanine, leucine and isoleucine. In those cases in which phenylalanine could be separated from leucine and isoleucine, it was observed that the changes had actually taken place in the leucine-isoleucine component of the spot. A decrease in the density of the spot representing liver glutamic acid and threo-

nine was also associated with lysine deficiency. The amino acid changes could not be correlated to any detectable degree to changes in the potassium content of the diet.

Because of its abundance in plant and animal tissues, potassium is usually not given consideration as a dietary adjunct in practical nutrition. The authors point out, out, however, that although primary potassium deficiency in humans may not exist commonly, problems involving potassium nutrition may occur as a result of disease or aberrant food supplies. J. D. L. Hansen and J. F. Brock (*Lancet* II, 477 (1954)) found that infants suffering from nutritional edema (some with kwashiorkor) had an increased need for potassium.

Moreover, potassium requirements seem to increase when tissue proteins are in process of repletion. The need for potassium in post-operative surgical cases as a protection against nitrogen loss has been stressed by L. P. Eliel, O. H. Pearson and F. C. White (*J. Clin. Invest.* 31, 419 (1952)) and by D. V. Frost and H. R. Sandy (*Proc. Soc. Exp. Biol. Med.* 83, 102 (1953)). The increased need for potassium by rapidly growing guinea pigs has been pointed out by P. Roine *et al.* (*Ibid.* 71, 90 (1949)) and H. R. Heinicke, A. E. Harper and C. A. Elvehjem (*J. Nutrition* 58, 269 (1956)).

## FACTORS CONTROLLING CHOLESTEROL EXCRETION

*In the hypercholesteremic rat, 4 per cent taurine reduced serum, liver, and aorta cholesterol significantly. In the hypercholesteremic rabbit, 2 per cent beta sitosterol reduced serum, liver, and aorta cholesterol but taurine and glycine had no significant effect.*

Experiments using C<sup>14</sup>-cholesterol have demonstrated that cholesterol is eliminated mainly by biliary excretion of bile acids (M. D. Siperstein, F. M. Harold, I. L. Chaikoff and W. G. Dauben, *J. Biol. Chem.* 210, 181 (1954)). G. A. D. Haslewood (*Physiol. Rev.* 35, 178 (1955)) and J. Bremer (*Biochem. J.* 63, 507 (1956)) point out that

bile acids are conjugated with taurine in the rat, with glycine in the rabbit and with either taurine or glycine in man or the monkey. The combination of diets high in cholesterol and deficient in sulfur-containing amino acids produces hypercholesteremia in the Cebus monkey, rat and mouse according to the report of G. V. Mann, S. B.

Andrus, A. McNally and F. J. Stare (*J. Exp. Med.* **98**, 195 (1953)) and L. C. Fillios and G. V. Mann (*Metabolism* **3**, 16 (1954)). The restoration of cystine or methionine, however, effected a significant reduction in the hypercholesteremia. Some cystine is converted to taurine in mammals (L. Eldjarn, *J. Biol. Chem.* **206**, 483 (1954)) and S<sup>35</sup>-labeled taurine is utilized for conjugation with bile acids (O. W. Portman and G. V. Mann, *Ibid.* **213**, 733 (1955)).

These results stimulated R. G. Herrmann (*Circ. Res.* **7**, 224 (1959)) to study the effect of taurine on hypercholesteremia resulting from cholesterol feeding. The effects of glycine and beta sitosterol were also studied.

The experimental animals were female rabbits weighing about 1.5 kg. and female albino rats weighing about 200 g. The animals were caged separately and food consumption was recorded. For the rabbits, commercial rabbit pellets were sprayed with the cholesterol and other supplements in an appropriate solution, while the rat diets consisted of pulverized chow mixed with the supplement in a mechanical mixer.

All animals were weighed weekly, and blood samples were taken for cholesterol determination. When the animals were killed at the end of the experiment, their tissues were examined both grossly and microscopically. Liver samples and entire aortas were frozen until cholesterol analyses could be performed.

In the rat experiment, all animals received a preliminary feeding for three weeks of a chow diet containing 2 per cent cholesterol and 1 per cent cholic acid. Group one was continued on the diet, while group two received the original diet, except that 4 per cent of taurine replaced an equal amount of chow. The diet for group three contained 4 per cent of glycine instead of taurine.

The serum cholesterol averaged 72 mg. per cent initially. However, during the first three weeks on the diet all serum cholesterol levels increased. Group one averaged 249 mg. per cent, group two

averaged 227 mg. per cent and group three averaged 307 mg. per cent. The group continuing the original diet showed a further increase in serum cholesterol to 312 mg. per cent during the fourth to seventh week. On the other hand, the average serum cholesterol level in taurine supplemented rats dropped to 202 mg. per cent while the glycine group with an average serum cholesterol level of 298 mg. per cent was essentially unchanged. In the period from eight to 11 weeks group one remained unchanged, group two showed a further drop to 180 mg. per cent and group three showed a small decrease to 279 mg. per cent.

Liver cholesterol averaged 6400 mg. per cent for group one with no taurine but was 2260 mg. per cent for group two supplemented with taurine (225 mg. per cent in normal rats). Similarly, the aorta cholesterol averaged 524 mg. per cent without taurine, but was 364 mg. per cent with taurine (152 mg. per cent in normal rats). No evidence of toxicity was seen either grossly or by microscopic examination of the tissues.

In the rabbit experiment, the preliminary diet was 96 per cent rabbit pellets, 1 per cent cholesterol, and 3 per cent cottonseed oil. After four weeks on this diet, four groups were formed of seven rabbits each. The preliminary diet was continued for group one, while for the other groups 2 per cent supplements of either taurine, glycine or beta sitosterol replaced an equal weight of rabbit pellets. These diets were fed for nine weeks, at which time the animals were killed for tissue cholesterol determination and pathological examination.

The initial serum cholesterol concentrations ranged from 47 to 55 mg. per cent, while four weeks after the preliminary diet was fed the average serum cholesterol levels ranged from 186 to 278 mg. per cent. After four weeks on the various experimental diets the serum cholesterol concentrations continued to increase, except in the group supplemented with beta sitosterol. The serum cholesterol in this group averaged

only 184 mg. per cent while the other values were 331 mg. per cent for the taurine group, 350 mg. per cent for the glycine group, and 374 mg. per cent for the control group one. After 13 weeks, the beta sitosterol supplement resulted in a cholesterol level of 233 mg. per cent, while the controls averaged 531 mg. per cent, the taurine group averaged 600 mg. per cent and the glycine group was 710 mg. per cent.

Thus, although variation was great among individual animals and from time to time, nevertheless the trends indicated no effect from taurine or glycine but a significant depression of cholesterol by beta sitosterol. This depressive effect was mirrored in cholesterol values of liver and aorta.

Cholesterol concentration in normal rabbits receiving no cholesterol supplement was 260 mg. per cent for the liver and 391 mg. per cent for the aorta. The control cholesterol-fed animals, on the other hand, had liver cholesterol values averaging 710 mg. per cent and aorta cholesterol averaging 1046 mg. per cent. Neither taurine nor glycine altered appreciably the deposition of cholesterol in either the liver or aorta.

However, the average liver cholesterol concentration of the beta sitosterol group was 465 mg. per cent and the average aorta concentration was 323 mg. per cent.

The most weight was gained by the control group, an average of 1243 g. in 13 weeks, while the group receiving the beta sitosterol gained the least, an average of 905 g.

Although the standard errors were very high (varying between 25 and 50 per cent, for example, in the rabbit serum cholesterol concentrations), the data indicate that serum and tissue cholesterol is lowered by beta sitosterol feeding.

The failure of either taurine or glycine to affect the cholesterol level in the rabbit is puzzling. Even if the mechanism of action of taurine in the rat is unrelated to cholic acid conjugation, a similar action might be expected in the rabbit. The results, therefore, suggest a fundamental difference in the control of cholesterol excretion between rabbits and rats. Further study of this phenomenon may reveal why rabbits fed cholesterol readily develop hypercholesterolemia and atherosclerosis while rats are much more resistant to this effect.

## DIET AND CHOLESTEROLEMIA IN THE RAT

*A diet promoting good growth in the rat, while leading to high serum cholesterol, contains 25 per cent saturated fat, 1 per cent cholesterol and 0.5 per cent cholic acid.*

The difficulties of carrying out dietary experiments with human subjects are obvious and, therefore, expediency rather than choice dictates the use of experimental animals. The commonest laboratory animal used is the albino rat, in which many of the nutritional disorders of man may be studied in at least a preliminary manner. There are several human nutritional diseases, however, to which the rat is not very susceptible. In particular, the rat does not appear to succumb naturally to many of the common disturbances of lipid metabolism. The disease of atherosclerosis, which is so universal in civilized man, has been induced in the chick,

rabbit and hamster by the use of high cholesterol diets (*Nutrition Reviews* 9, 295 (1951); 10, 148 (1952)), while the dog and rat are more refractory to this treatment.

The assumption that the degree of atheromatosis is correlated with hypercholesterolemia has led many workers to use this latter criterion as the end result of their dietary manipulations. Hypercholesterolemia, however, is not readily achieved in the rat, nor does it appear to be correlated directly with atheromatosis (*Nutrition Reviews* 11, 152 (1953); 12, 220 (1954)). Nevertheless, a high fat diet with additional cholesterol and bile salts has been used to achieve both hyper-



cholesterolemia and atheromatous deposits in the rat, particularly if the animals are first rendered hypothyroid either with radioiodine or with thiouracil (*Ibid.* 11, 152 (1953)). However, to facilitate the use of the rat as an experimental animal for this disease, more knowledge of the dietary conditions leading to hypercholesterolemia, with or without atheromatous deposition, is necessary.

Such an attempt has been reported by N. Nath, R. Wiener, A. E. Harper and C. A. Elvehjem (*J. Nutrition* 67, 289 (1959)) by taking into consideration such factors as dietary saturated and unsaturated fat, pyridoxine deficiency, cholesterol, cholic acid and thiouracil.

Male weanling rats were maintained on a diet consisting of 20 per cent casein (supplemented with 2 per cent DL-methionine), 4 per cent salts and adequate vitamin supplements, with the remainder consisting of sucrose and hydrogenated coconut oil.

In the first experiment, it was found that growth at five and ten weeks was greater with 10 per cent than with 1 or 25 per cent hydrogenated coconut oil. Apparently the rats were suffering from an essential fatty acid deficiency promoted by the high content of saturated fat (H. J. Deuel, Jr., *et al.*, *J. Nutrition* 55, 337 (1955)), since growth was normal and the dermatosis which developed was prevented when 1 per cent corn oil was included in the diet. The serum cholesterol was not affected by increasing the level of the dietary hydrogenated coconut oil. However, the addition of 1 per cent dietary cholesterol increased hypercholesterolemia about fourfold (to 336 mg. per cent), while the addition of 1 per cent corn oil in the presence of 1 per cent dietary cholesterol lowered this latter value to 209 mg. per cent. The liver lipids and liver cholesterol were greatly increased by dietary cholesterol, as would be expected.

In the second experiment, the effects of pyridoxine deficiency were studied. Very poor growth and severe dermatosis were ob-

served after 15 weeks on a diet deficient in pyridoxine but including 25 per cent hydrogenated coconut oil and 1 per cent cholesterol. Corn oil alleviated the dermatosis, but pyridoxine was necessary to bring about normal growth. As before, serum and liver cholesterol values were elevated by dietary cholesterol.

In the third experiment, the additional effects of 1 per cent cholic acid and 0.3 per cent thiouracil were tested. When the cholic acid was added to a diet containing 25 per cent hydrogenated coconut oil and 1 per cent cholesterol, weight gain was somewhat reduced while the serum and liver cholesterols were increased two to threefold at the end of 12 weeks. Thiouracil increased the serum cholesterol an additional threefold, but actually lowered liver cholesterol and severely depressed growth.

Corn oil had some serum cholesterol-lowering effect in the presence of cholic acid, but did not influence liver cholesterol. In those diets where thiouracil was present, corn oil had no effect. It is unfortunate that the use of higher levels of corn oil was not attempted since, in most studies, little cholesterol-lowering effect is noted until the unsaturated fat becomes a sizable proportion of the total diet.

In the fourth experiment, an attempt was made to ascertain the optimum proportions of cholic acid and cholesterol for production of high serum cholesterols without undesirable side effects such as diarrhea. It was decided that pyridoxine and thiouracil should not be excluded from the diet since deficiencies of these favor anorexia and unfavorable tissue changes. Both dietary cholesterol and cholic acid induced hypercholesterolemia with the cholic acid having the greater influence. The highest serum cholesterol values were obtained when 0.75 per cent of each factor was included in the diet, but some diarrhea was present.

Nath and his co-workers therefore propose, on the basis of these studies, that an experimental diet promoting both good

growth and a significantly high serum cholesterol level (from about 80 to 450 mg. per cent in three weeks) contains 25 per cent hydrogenated coconut oil, 1 per cent cholesterol and 0.5 per cent cholic acid.

The authors state that variations in the fat content of the diet had little effect on the serum cholesterol of the rats. The high levels of hydrogenated coconut oil were used primarily in order to promote an essential fatty acid depletion since it has been speculated that the essential fatty acids may be important in regulating the serum cholesterol level (H. M. Sinclair, *Lancet* **I**, 381 (1956)). An explanation for the action of cholic acid is that it promotes cholesterol absorption resulting in higher levels of serum cholesterol and serum lipid phosphorus as well as deposition of cholesterol in the liver. Histological examination of the cardiovascular system was not carried out in this series of studies.

It seems likely that these observations may be valuable in the formulation of useful diets for studying serum and liver cholesterol changes in the rat. However, there are several indications that studies in the rat cannot be directly applicable to the human disease. First, the rat does not develop a significant spontaneous atherosclerosis and it is necessary to employ relatively severe dietary conditions. Second, dietary fat seems to have less influence on the serum cholesterol in the rat than in man (*Nutrition Reviews* **13**, 8, 44, 138 (1955)). Finally, dietary cholesterol appears to be effective in promoting hypercholesterolemia in the rat while there is no conclusive evidence that this sterol influences human cholesterol levels (*Nutrition Reviews* **14**, 327 (1956)).

Therefore, despite the many limitations for experimental study, the ultimate evaluation must be done on man, although preliminary data may be obtained with experimental animals such as the rat.

## VITAMIN D AND INTESTINAL ABSORPTION OF RADIOCALCIUM

*Active transport of  $\text{Ca}^{45}$  has been demonstrated using everted sacs of small intestine. Transport through sacs prepared from rats fed a vitamin D-deficient diet was depressed.*

For many years it has been known that vitamin D influences the intestinal absorption of calcium (*Nutrition Reviews* **13**, 271 (1955); **16**, 148 (1958)), but relatively little is known about the mechanism of this effect. Most studies of the problem have been made using *in vivo* techniques and, although these studies have clearly shown a need for vitamin D for normal calcium absorption, they have provided little information about the nature of the interrelationships involved in the absorption process.

D. Schachter and S. M. Rosen (*Am. J. Physiol.* **196**, 357 (1959)) have approached the problem through *in vitro* methods, using everted sacs from segments of rabbit, rat, and guinea pig intestine. Segments of

the upper small intestines of 24-hour-fasted animals were taken, washed in cold saline, cut into short lengths, and formed into closed sacs. The sacs were filled with a standard medium containing  $\text{Ca}^{45}$  and the same medium was used to bathe the outside of the sacs. They were placed in Warburg flasks and allowed to respire for three hours at 37°C under an atmosphere of oxygen.

At the end of the incubation period the inside (serosal) and the outside (mucosal) solutions were collected and the  $\text{Ca}^{45}$  concentration of an aliquot of each solution was determined. Tissue uptake of  $\text{Ca}^{45}$  was estimated by subtracting the final  $\text{Ca}^{45}$  content of the medium from the initial  $\text{Ca}^{45}$  content of the medium. The validity of the



estimation of tissue uptake was verified by ashing the tissue in nitric acid, precipitating the calcium as the oxalate, and determining the  $\text{Ca}^{45}$  content of the precipitate.

A measure of the extent of active transport was obtained by calculating the  $\text{Ca}^{45}$  concentration ratio, I/O, between the inside and outside media. Ratios for I/O greater than 1, indicating transfer against a concentration gradient, were taken as evidence of active transport of  $\text{Ca}^{45}$ . In a large number of tests with preparations from normal animals, ratios of from 3 to 5 were obtained. The process of active transport could be shown in this system to require the simultaneous occurrence of oxidative metabolism since the replacement of oxygen by nitrogen, reduction of the incubation temperature to  $5^{\circ}\text{C}$ , or the presence of sodium fluoride depressed the I/O ratios to values not exceeding 1.0. Tissue uptake occurred, however, despite the inhibition of oxidative metabolism, possibly owing to exchange reactions of  $\text{Ca}^{45}$  with bound calcium in the tissues.

Various metabolites including amino acids, tricarboxylic acid cycle intermediates, fatty acids and carbohydrates were tested for their influence on the transport process. Only glucose, fructose, and maltose appeared to stimulate  $\text{Ca}^{45}$  uptake. Glucose and maltose increased the amount of transport to double that of the control without substrate, and fructose increased it even more. Citrate and oxalate were inhibitory.

Three groups of compounds with inhibitory action were tested. The presence of 2,4-dinitrophenol completely inhibited  $\text{Ca}^{45}$  transport, although oxygen consumption was increased by 20 per cent, suggesting that (in common with other active transport mechanisms) the transport of  $\text{Ca}^{45}$  is dependent on the production of high energy phosphate bonds. Several divalent cations were tested as possible competitive inhibitors of calcium transport. Magnesium and cobalt ions, in contrast to strontium and barium ions, produced a consistent

depression in the amount of  $\text{Ca}^{45}$  transport. The sodium salts of versene, glucuronic acid, and citric acid, all chelating agents for calcium, inhibited the transport of  $\text{Ca}^{45}$ .

Intestinal segments from rabbits and rats maintained on a rachitogenic diet were less effective in transporting  $\text{Ca}^{45}$  than were similar segments from animals maintained on the same diet but supplemented with vitamin D. A depression in  $\text{Ca}^{45}$  transport could be demonstrated in intestinal segments from rats within two days after the animals had been given a rachitogenic diet.

This stimulated the authors to examine the possibility that the inhibition of citrate oxidation by vitamin D (H. F. De Luca *et al.*, *J. Biol. Chem.* **224**, 201 (1956)) and the consequent accumulation of a calcium-citrate complex in the serosal fluid might be essential for the active transport of  $\text{Ca}^{45}$  and for the effect of vitamin D on calcium transport. From a knowledge of the citrate concentration in the medium and as a result of calculations based on the pK value for the calcium citrate complex, the authors concluded that active transport of calcium cannot be mediated through a calcium-citrate complex and that the effect of vitamin D on citrate oxidation cannot be responsible for its effect on calcium absorption.

Although the information obtained by the authors provides evidence that calcium is actively transported by the small intestine through a mechanism that is dependent on oxidative phosphorylation and dietary vitamin D, the importance of active transport of calcium *in vivo* cannot be evaluated from the results of these *in vitro* experiments. Since the active transport mechanism can transport calcium against a calcium concentration similar to that in plasma, the authors conclude that the participation of active transport *in vivo* is possible. Transport by passive diffusion in the intact animal, however, could occur if there were a calcium concentration

gradient from the lumen of the small intestine to the bloodstream.

Also the experiments do not provide evidence about the way in which vitamin D influences calcium absorption. It is of considerable interest, however, that a fall in the extent of active transport of calcium could be detected using sacs that were prepared from rats fed a vitamin D-deficient diet. The activity could not be restored by

adding calciferol in propylene glycol directly to the medium. This suggests, but is not of course conclusive evidence, that the effect of vitamin D is indirect. The possibility that an *in vitro* system in which vitamin D is active might be obtained by some method of preincubating the sacs from rachitic animals with vitamin D would appear to be worthy of further investigation.

### TOCOPHEROL DERIVATIVES IN EXPERIMENTAL DYSTROPHY

*Alpha-tocopherol, its acetate, hydroquinone and hydroquinone disuccinate cure experimental dystrophy if administered orally. Tocopherol and the hydroquinone are effective intravenously but the esters are not.*

The relationships between alpha-tocopherol and its derivatives and muscular dystrophy in man and experimental animals are not clear (*Nutrition Reviews* 3, 259 (1945); 4, 324 (1946)). Since in the latter, the disease can be produced by vitamin E-deficient diets, often with peroxidized oil, it should be possible to delineate exactly the conditions under which various tocopherol derivatives will present or alleviate the typical symptoms such as creatinuria and observable muscular lesions.

However, although oral administration of alpha-tocopherol was shown to cure experimental dystrophy in rabbits and rats and the tocopherol hydroquinone was found to be effective when given intravenously, the hydroquinone appeared to have much less effect when given orally to hamsters (W. T. West and K. E. Mason, *Am. J. Phys. Med.* 34, 223 (1955)), and no effect when fed to children (P. L. Harris and K. E. Mason, *Am. J. Clin. Nutrition* 4, 402 (1956)). Esters of the hydroquinone, which should be readily hydrolyzed in the body, also appeared to be ineffective.

In order to clarify these observations, J. B. Mackenzie and C. G. Mackenzie (*J. Nutrition* 67, 223 (1959)) fed young rats a vitamin E-deficient diet containing 10 per cent lard which had been heated to destroy

antioxidants. After 20 weeks on the diet, the experimental rats had gained 20 per cent less weight than the controls with about seven times the creatine excretion and some muscular lesions. Although single 5 mg. oral doses of alpha-tocopherol acetate reduced the creatinuria within three days and muscles became normal at the end of two weeks (after a total of 20 mg. of the acetate), the disuccinate of alpha-tocopherol hydroquinone had little effect, even after a two-week period of daily intravenous injections.

When dystrophic rats were given daily injections of either alpha-tocopherol or alpha-tocopherol acetate, it was found that the acetate was much slower in effecting a diminution of creatinuria or muscle lesions even though it had been equally effective when given orally. Under the same conditions, the hydroquinone appeared to be as effective as the alpha-tocopherol. However, the hydroquinone disuccinate, injected at four times the molar level of the tocopherol, was almost without effect, although it was as effective as alpha-tocopherol or the hydroquinone when given orally to dystrophic rabbits.

Thus it appears that both alpha-tocopherol and the hydroquinone can cure experimental dystrophy when given by injection.

The esters of these compounds, on the other hand, although effective by mouth, are apparently hydrolyzed too slowly in the blood to be very effective on injection. The

authors point out that either the free  $\alpha$ -tocopherol or the hydroquinone might be used in clinical cases in which a rapid raising of the blood level is desired.

### EFFECTS OF THIOURACIL AND SITOSTEROL ON DIET-INDUCED HYPERCHOLESTEROLEMIA AND LIPOMATOUS ARTERIAL LESIONS IN THE RAT

*Atherosclerotic lesions were induced in rats fed a semisynthetic diet plus thiouracil. Addition of sitosterol to this regimen protected most rats. Sitosterol interfered with absorption of cholesterol and perhaps of thiouracil.*

Although the rat is quite resistant to the development of hypercholesterolemic atherosclerosis, recently devised techniques have enabled investigators to produce this condition in them (*Nutrition Reviews* 11, 152 (1953); 13, 20 (1955); 15, 141 (1957); and W. S. Hartroff and W. A. Thomas, *J. Am. Med. Assn.* 164, 1899 (1957)). A combination of an atherogenic diet containing large amounts of saturated fatty acids and cholesterol together with thiouracil would produce arterial lesions similar to the human atherosclerotic lesion.

Sitosterol has been reported to protect hypothyroid rats from these atherogenic changes (M. M. Best and C. H. Duncan, *Circulation* 14, 344 (1956)).

Best and Duncan (*Am. Heart J.* 58, 214 (1959)) studied the effects of both thiouracil and sitosterol upon rats fed the same atherogenic diet. Twenty-four male rats were divided into four groups of six each. The first group was fed laboratory chow and the second was fed the semisynthetic diet. Group three was fed this diet plus thiouracil. Group four was fed the diet plus thiouracil and sitosterol. These regimens were continued for fifteen weeks and then the animals were killed. Measurements were made of their serum cholesterol, hepatic cholesterol, hepatic weight, thyroid weight, and of the microscopic changes in their hearts and kidneys.

The results showed that the first two groups of animals ate well and gained

weight, but groups three and four ate poorly and did not gain after the first few weeks. The serum cholesterol was lowest in the control group, higher in the group fed a special diet, extremely high in the group fed this diet plus thiouracil, and somewhat lower in the group fed sitosterol. The hepatic concentration of cholesterol, however, was highest in group two, lower in group three and still lower in group four. Hepatic weight reflected this storage of cholesterol. The thyroid glands were grossly enlarged in the group fed thiouracil, less so in the group fed sitosterol and thiouracil.

Histologic sections of heart and kidneys were stained for fats and disclosed deposits in none of group one, one heart of group two, three hearts and four kidneys of group three, and only two hearts of group four. In three rats of group three, renal infarcts were found.

The authors pointed out that sitosterol behaves in a fashion similar to cholesterol, and hence might give falsely elevated values for serum cholesterol. Also they questioned whether sitosterol might interfere with gastrointestinal absorption of thiouracil, since the thyroids of animals so treated were not as enlarged as those from animals fed thiouracil alone.

They wisely considered their groups of animals to be too small for firm conclusions. However, it appeared that hypercholesterolemic atherosclerosis occurred less frequently in rats fed sitosterol along with an

atherosclerotic ration than in those fed this ration alone. The mechanism of this effect could be interference with absorption of cholesterol or interference with absorption of thiouracil, or both.

In this preliminary report, the authors have given further evidence for dietary and hormonal regulation of the metabolism

of cholesterol, and secondarily, for the occurrence of atherosclerosis. It would seem advisable to determine whether sitosterol exerts a similar effect in thyroidectomized rats fed an atherogenic diet. Also pair feeding should be considered, since the first two groups gained weight while the second two did not.

### CHOLESTEROL ABSORPTION IN THE RAT

*Bile salts are generally considered to facilitate the absorption of cholesterol, but the observation that bile salts inhibit cholesterol absorption in vitro suggests that these compounds may exert other effects.*

The presence of bile acids in the intestine leads to higher concentrations of cholesterol in lymph, liver and blood, an effect that is generally attributed to increased intestinal absorption of cholesterol. It has been suggested that pancreatic cholesterol esterase and fatty acids are also involved in cholesterol absorption (*Nutrition Reviews* **13**, 246 (1955)), but neither the exact mechanism of cholesterol absorption nor the specific role of bile acids in relation to the effects of these other factors is clearly understood.

In a study of the role of bile acids in cholesterol absorption, *in vitro* experiments on cholesterol uptake by inverted (more correctly everted) sacs of rat intestine have been carried out by A. L. Smith, R. Hauk and C. R. Treadwell (*Am. J. Physiol.* **193**, 34 (1958)). They used small intestines from fasted male rats, weighing 200 to 300 g. The sacs from one intestine were randomly distributed among control and experimental groups. The basic external medium was a 0.154 molar phosphate buffer (pH 6.2) and contained per 4 ml., 0.0205 micromoles potassium chloride, 0.0154 micromoles calcium chloride, 0.0051 micromoles manganese sulfate, 120 mg. glucose and 20 mg. of purified human or bovine serum albumin. Cholesterol, oleic acid and sodium taurocholate were incorporated into the external medium by homogenization. The internal

(serosal) medium was 0.5 to 1.0 ml. of the basic external medium prepared without glucose. The sacs were incubated in 4 ml. of external medium in a Dubnoff shaker under either 100 per cent oxygen or 95 per cent oxygen and 5 per cent carbon dioxide.

The incubation of such sacs in emulsions of cholesterol, either free or esterified, led to the disappearance of cholesterol from the external medium and an accumulation of free or esterified cholesterol in the sacs and against a concentration gradient. The total cholesterol content of the sacs before incubation was 1.8 to 2.4 mg. per g. while the external medium contained 1 mg. per ml. After incubation the total cholesterol content of the sacs was 4.8 to 5.4 mg. per g.

The uptake of cholesterol by sacs from various segments of the intestine from the lower duodenum to the upper ileum was constant and was independent of the pH of the external medium over the range from 5.9 to 7.2. Bile salts added to the external medium containing cholesterol inhibited cholesterol uptake, the inhibition being 97 per cent when the molar concentration of taurocholate was four times that of cholesterol. The addition of oleic acid to the medium containing cholesterol also decreased cholesterol uptake. The inhibition was less in the presence of both bile salt and oleic acid. Addition of an extract from

rat pancreas to the external medium also depressed the uptake of cholesterol.

In these studies, large variations were observed among different animals and the results were primarily of a qualitative nature. The variability was reduced in later experiments (Smith and Treadwell, *Am. J. Physiol.* **195**, 773 (1958)) in which some of the factors involved in cholesterol uptake by inverted intestinal sacs were studied quantitatively. The conditions suggested for getting reproducible results are the use of (a) 200 to 250 g. male rats, (b) animals fasted 24 hours, (c) 3.0 cm. sacs, (d) a one hour incubation, (e) sacs not rinsed with saline after incubation.

Employing these sacs, the authors showed that glucose is not required in the medium for cholesterol uptake. The uptake of cholesterol (10 micromoles in the external medium) was 5.5 micromoles in the absence of glucose in the medium. The uptake of cholesterol when the medium contained 0.3 or 3 per cent of glucose was 6 micromoles. Results obtained with sacs incubated in a medium containing 10 micromoles of cholesterol and 0 to 120 mg. of albumin showed that albumin is not required for cholesterol absorption, but that albumin does facilitate cholesterol uptake.

Further, the presence of bile acids in the external medium was not obligatory for cholesterol uptake by the sacs. In fact, both conjugated and unconjugated bile acids inhibited cholesterol uptake, marked inhibitions being obtained with cholic and dehydrocholic acids, taurocholate and glycocholate. Purified samples of taurocholate and glycocholate were less inhibitory than commercial samples, suggesting that the latter may contain an inhibitor.

To study the mechanism of this inhibition, sacs were preincubated in an external medium containing 20 micromoles of taurocholate for various lengths of time or with different concentrations (0 to 80 micromoles) of taurocholate for one hour, and later with the medium containing

cholesterol for one hour. As the taurocholate concentration of the pre-incubation medium was increased, the cholesterol uptake decreased. Cholesterol uptake also decreased as the period of pre-incubation with taurocholate increased.

These findings, according to the authors, suggest that the inhibitory effect of taurocholate is in the sac rather than in the medium, and that taurocholate is taken up slowly in the sac where it inhibits cholesterol uptake. Also, they pre-incubated sacs with taurocholate and then incubated them in media containing different concentrations of cholesterol and concluded that taurocholate inhibits cholesterol uptake nonreversibly.

Some of these observations are not in agreement with the results of other studies on whole animals in which lymph-cholesterol concentration was taken as a measure of cholesterol absorption. Thus G. V. Vahouny and Treadwell (*Am. J. Physiol.* **191**, 179 (1957)) reported that administration of albumin in emulsions containing cholesterol, oleic acid and taurocholate to lymph-fistula rats lowered the amount of cholesterol found in the lymph in a 24 hour period.

Also, Vahouny, C. H. Woo and Treadwell (*Am. J. Physiol.* **193**, 41 (1958)) found that in lymph-fistula animals a molar relationship of oleic acid, taurocholate and cholesterol of 8 to 4 to 1 was most effective for lipid and cholesterol absorption from the intestine. They also observed that in rats with bile fistulas and lymph fistulas, lipid and cholesterol absorption was low, and an intragastric administration of taurocholate along with cholesterol and fatty acid failed to cause any appreciable elevation of lymph-cholesterol concentration. These results suggested that a continuous supply of bile and the presence of bile acids, fatty acids and cholesterol is necessary for optimal cholesterol absorption.

The presence of bile acids or fatty acids is not obligatory for cholesterol uptake from external medium by inverted intestinal

sacs. In fact, these substances were shown to inhibit cholesterol uptake. This evidence, according to the authors Smith and Treadwell (*Am. J. Physiol.* **195**, 773 (1958)) suggests that the solubilizing effect of bile acids is not required for cholesterol entrance into mucosal cells. They have suggested that bile acids may be required by the mucosal cells for the formation of cholesterol esters prior to the entrance of esters into the lacteals or that they may play some part in the formation of chylomicrons.

A. Pihl (*Acta Physiol. Scandinav.* **34**, 206 (1955)) has demonstrated that bile acids at a level of 0.5 per cent in the diet have little effect on the rate of cholesterol absorption, and has suggested that a reduced rate of cholesterol catabolism combined with a normal rate of cholesterol absorption would account for cholesterol accumulation in the liver in the presence of dietary bile acids. W. T. Beher and G. D. Baker (*Proc. Soc. Exp. Biol. Med.* **98**, 892 (1958)) have presented evidence to show that dietary cholic acid retards the rate of mobilization of liver and serum cholesterol.

Since glucose is not required in the incubation medium for the uptake of cholesterol by inverted intestinal sacs, active transport of cholesterol must require a minimum

expenditure of energy. Smith *et al.* (*loc. cit.*) explain that this may be possible because of the binding of cholesterol to a cellular protein immediately after entrance into the epithelial cells as previously suggested by J. Glover and C. Green (*Biochemical Problems of Lipids*, p. 359. Edited by G. Popjak and E. Lebreton. Interscience, London, 1956).

The facilitation of cholesterol uptake by the presence of albumin was taken to suggest that albumin may facilitate cholesterol transfer to the acceptor protein on the cell walls. The possibility of acceptor protein was suggested by the separation of a cholesterol protein complex from the washings of the intestine. They proposed that the entrance of cholesterol into the epithelial cells of the mucosa is mediated by a series of transfers of cholesterol from one protein to another. The inhibition of cholesterol absorption by bile acids, according to Smith *et al.*, could be due to non-reversible binding of bile acids at the active sites of these proteins.

The results of these studies emphasize the gaps in our knowledge of the mechanism of cholesterol absorption and the need for further work on this subject.

## PROTEIN, ENERGY AND POTASSIUM

*Increases in both protein content and energy content of the feed increase the requirements of chicks for potassium. With increasing age, the chick's requirement for potassium is lessened.*

Several investigators (B. Ben Dor, *Proc. Soc. Exp. Biol. Med.* **46**, 341 (1941); M. B. Gillis, *J. Nutrition* **36**, 351 (1948); **42**, 45 (1950); C. H. Burns, W. W. Cravens and P. H. Phillips, *Ibid.* **50**, 317 (1953)) have demonstrated the necessity for including potassium in the diet of young chicks. M. B. Gillis (*loc. cit.*) found that a higher quantity of potassium was required when the phosphorus content of the diet was borderline or suboptimal, and that potas-

sium is required for proper bone formation. Burns, Cravens and Phillips (*loc. cit.*) concluded that the requirement was in part dependent upon the growth rate of the chicks. Sodium and potassium were found to be toxic if one of these minerals was fed greatly in excess of the other.

Results obtained by R. M. Leach, Jr., R. Dam, T. R. Zeigler and L. C. Norris (*J. Nutrition* **68**, 89 (1959)) have further suggested an increased need for potassium

when a high-protein or high-energy diet is fed.

The composition of the basal diet employed by these workers was developed by R. Dam *et al.* (*Poultry Sci.*, **36**, 1110 (1957)) for use in studies of unidentified chick factors. It consisted of 45.1 per cent glucose, 10.0 per cent corn oil, 33.3 per cent purified soybean protein, 3.23 per cent cellulose, 0.84 per cent DL-methionine, 0.36 per cent glycine, 5.84 per cent salt mixture, 1.25 per cent vitamin mixture, and 0.02 per cent butylated hydroxytoluene. Duplicate lots of 13 to 15 one-day-old Vantress X White Plymouth Rock male chicks were used in all experiments.

Preliminary results indicated that the basal diet was borderline in mineral content. Three experiments were, therefore, conducted in which the effect of increasing the sodium and potassium content was ascertained. Increasing the sodium from 0.26 to 0.33 per cent and the potassium from 0.27 to 0.50 per cent resulted in consistent increases in growth rate. The mineral responsible for this increase seemed to be potassium. When graded levels of potassium were fed, the results indicated that the potassium requirement for optimum growth was approximately 0.30 per cent, while 0.20 per cent was required to prevent excessive mortality.

The potassium requirement of young chicks on the basal diet was determined to be somewhat more than 0.27 per cent (0.27 per cent was the actual potassium content of the basal diet), and experiments were carried out to study the effect of dietary factors such as protein and energy content on this potassium requirement. In one experiment, diets containing 25, 30 and 37 per cent protein were fed. The diets were kept isocaloric at approximately 3.68 calories per gram by varying the fat content. When the level of potassium was inadequate, increasing the protein content from 25 to 37 per cent of the diet markedly depressed growth rate and increased mortality. How-

ever, increasing the protein level had no effect on the chicks which received sufficient potassium to achieve an optimum growth rate.

P. R. Cannon, L. E. Frazier and R. H. Hughes (*Metabolism* **1**, 49 (1952)) have reported that potassium is necessary for maintenance of nitrogen balance in rats. M. Iacobellis, E. Muntwyler and C. L. Dodgen (*Am. J. Physiol.* **185**, 275 (1956)) have shown that potassium is involved in the maintenance of certain free amino acids in cellular tissue. These results all indicate a function of potassium in protein metabolism, especially catabolism, since the higher levels of protein are probably in excess of the animals' needs.

In another experiment, diets containing from 3.35 to 4.25 calories of metabolizable energy per gram were fed. Potassium levels varying from 0.15 to 0.45 per cent were included in the 3 per cent corn oil diet, while the graded levels in the 10 and 20 per cent corn oil diets were increased according to increases in the energy content of the diet. This resulted in a constant ratio of potassium to energy for each potassium level. The results of this experiment indicated that the potassium requirement is related to the energy content of the diet rather than to the fat content per se. In the diets in which potassium was the limiting factor, the grams of gain per gram of potassium was markedly uniform at each level of potassium regardless of the fat content of the diet.

To further confirm this observation, an analysis of variance showed that highly significant growth increases ( $P < 0.01$ ) were obtained with all diets by increasing the potassium content, but no interaction was revealed between the level of fat and the level of potassium. An improvement in growth was also obtained by increasing the fat content from 3 to 10 per cent. With increasing age, the chick's requirement for potassium was observed to decrease.



## NOTES

## Letters to the Editor

Dear Sir:

I was very interested that a recent article in *Nutrition Reviews* emphasized the role of protein in the development and treatment of some of the anemias we have in Ghana (*Nutrition Reviews* 17, 193 (1959)).

Dietary iron deficiency is not at all common in this area and it is felt that the terms hypochromic anemia and "iron deficiency anemia", which was rightly put into quotes, should not be used interchangeably. Clinicians and field workers here as elsewhere in the tropics recognize the fact that huge loads of hookworm may exist without any serious anemia and the clinical severity of such an infection depends on the state of nutrition of the individual. In Ghana this often means the extent of protein malnutrition present.

It is my experience that removal of the hookworms and iron therapy alone never achieve fully successful results. For outpatient therapy such patients always benefit from an addition of skimmed milk to the diet. This agrees with the findings of Stanier and Holmes (*Brit. J. Nutrition* 8, 155 (1954)). The role of protein in the treatment of hookworm anemia has not been separately recognized in the past because reporters have ignored the drastic improvement in the diet of such a patient when he goes onto hospital food.

In this area, protein malnutrition and other nutritional deficiencies take a severe toll of infant and child life. In the first year of life, malaria is probably more important since the infants are often breast fed satisfactorily. Bruce Chewatt halved the infant mortality in Kilaro by controlling malaria.

Among the small children of one to four, however, the situation is different. This is the age during which some immunity to malaria has already been established. Malnutrition, which in Ghana means protein

malnutrition, is the usual primary or secondary cause of death. From the regions where data exist, the ratio of deaths of children aged one to four to that of infants is between 0.6 and 0.8 (1957). The figure for Britain has dropped from 0.66 in 1870-72 to 0.155 in 1955. This ratio has been suggested by Wills and Watkins (1958), quoted by Platt as a good index of malnutrition for communities where vital statistics are not good. Post mortem findings in Accra certainly support the suggestion that malnutrition is one of the most important causes of death in young children.

The relationship of protein malnutrition to anemia in tropical regions has been excellently dealt with by Platt (*Proc. Nutr. Soc.* 15, 103 (1956)).

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Dear Sir:

We were interested in the views recently expressed on the etiology and treatment of iron deficiency anemias in Ghana (*Nutrition Reviews* 17, 193 (1959)).

Although we have no experience in Ghana, we should be surprised if the iron deficiency anemias there are in any way different from those described in nearly all other parts of the tropical world and which have been shown again and again to respond to oral iron administration and in most cases to be associated with considerable intestinal blood losses due to hookworm infection, which can reach 200 or more ml. per day with worm loads of up to 2500 adult worms, of which 40 to 70 per cent are *Ancylostoma duodenale*. Blood and iron losses have been estimated using radio-isotope techniques (Roche *et al.*, 1957; Foy and Kondi, 1958, in press 1959).

These blood losses stop with the removal of the hookworms. Treatment with oral iron results in a rise in the hemoglobin level to

normal in the majority of cases. Removal of the hookworms will not result in any improvement of the blood picture unless iron is also given. This is to be expected since removal of the worms is unlikely to replace exhausted or diminished iron stores. Unless, however, the worms are removed, anemia will sooner or later recur once iron therapy is stopped. The marrows in these iron deficiency anemias are erythronormoblastic, active and sometimes have giant stab-cells, the significance of the latter not being fully understood (Foy and Kondi, in press 1959). These anemias have been shown to be typically iron deficient, having low mean corpuscular hemoglobin concentrations, absence of marrow iron, high unsaturated iron binding capacities and low serum iron, and hemoglobin values in most cases between 2 and 8 g. per 100 ml.

These anemias are due to a specific deficiency of iron, and it is to be expected that they would respond to iron. In no instance have we found them to respond to protein alone. In rare cases there may be a slightly more rapid response to iron and protein combined; so far as we know there is no evidence that iron deficiency anemias respond to protein alone.

Regarding the mortality among children in Ghana, is not this to be anticipated in an area of high incidence of malaria and in an age group which has not yet acquired the "immunity" characteristic of the adult African who has been exposed to malaria?

The anemia associated with acute or chronic malaria is not iron deficient and responds in the great majority of cases to anti-malaria treatment unless associated with hookworms, in which case iron will also be necessary.

Other factors may also enter into the etiology of iron deficient anemias in the tropics such as deficiency of iron in the diet, poor absorption of iron on account of the bulk and high phytate and phosphate and low calcium content, which may adversely affect absorption. Excessive dermal losses

associated with high perspiration and cell desquamation rates may also be a factor.

If it can be established that the Ghana iron deficiency anemias do indeed respond to dried skimmed milk, we should like to be assured that the milk really had no iron in it. Walker (1956) has shown that food and fermented liquors prepared in iron utensils have a very high iron content, and we should like to know whether the skimmed milk taken in Ghana was prepared in iron utensils and whether it had any iron in it.

We feel that it is imperative to emphasize that the iron deficiency anemias of the tropics are most unlikely to respond to the administration of dried skimmed milk which is devoid of iron, as suggested in your article.

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#### Recent Books

*Human Nutrition and Dietetics.* Sir Stanley Davidson, A. P. Meiklejohn and R. Passmore. Foreword by Lord Boyd Orr. E. & S. Livingstone Ltd., Teviot Place, Edinburgh. Pp. 856. Price 84s.

*Food. The Yearbook of Agriculture.* U.S. Department of Agriculture. For sale by the Superintendent of Documents, Washington 25, D.C. Pp. 736. Price \$2.25.

*History of the American Dietetic Association 1917-1959.* Edited by Mary I. Barner. Published by J. B. Lippincott Co., Philadelphia, Pa., and Montreal, Canada. Distributed in Great Britain by Pitman Medical Publishing Co., Ltd., London. Pp. 312.

#### Erratum

In the August 1959 issue under Recent Books the number of pages given for *Proteins in Foods* by S. Kuppaswamy, M. Srinivasan and V. Subrahmanyam (Indian Council of Medical Research) should have been 290.

# NUTRITION REVIEWS

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## POULTRY NUTRITION RESEARCH

In recent years, a remarkably short time has elapsed between the discovery of new information and its application in applied poultry nutrition. This reversal of the usual state of affairs, in which scientists have had to convince non-scientists of the merit of their findings, has found feed manufacturers who read trade magazines using new facts several months before they have appeared in scientific journals.

This ever-present pressure of fact application has had mixed results. In its favor has been its success in achieving efficient production of poultry meat and eggs. In opposition to it, however, is its diversion of many eager minds from imaginative paths of investigation to more pedestrian ones. This has been particularly evident in recent years, when, for example, the headlong search for unidentified growth factors has involved many more investigators than would seem to be justified, even on the practical grounds of improving the quality of feeds.

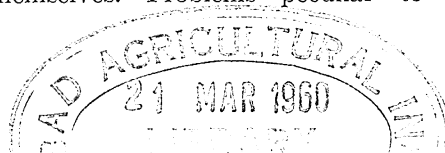
As the interest in applied nutrition has surged from one popular topic to another, many worthwhile studies have been virtually abandoned. This has been particularly noticeable when natural food sources of a nutrient can be supplemented economically by a manufactured product. Some examples of this are: (1) The importance of manganese in poultry diets was discovered over 20 years ago but for the last 15 years almost no research has been done to uncover its nutritional significance; it is no longer a practical problem. (2) The mode of action of antibiotics in stimulating growth is perhaps a less clear-cut example, but there has been a noticeable decrease in interest in determining how antibiotics work, now that their practical usefulness has been evaluated. (3) Several vitamins and min-

erals have not received enough attention because the practical problem of diet sources has been solved.

This introduction to the present status of poultry nutrition may seem to be an unduly critical one, but it is certainly not meant to be. In the past, poultry nutritionists have often led those working with other animals into new areas of discovery. We need to continue maintenance of a balance between research that may have future usefulness (fundamental research) and that which may be used immediately (applied or practical research).

*Chemically defined diets.* One measure of success in nutrition is the effectiveness of purified or completely defined diets in replacing foods of natural origin. Poultry nutritionists have gone far toward reaching this goal, and sufficient information is now available to permit good growth and reproduction of birds fed purified diets. Diets in which all components are of known composition (defined or synthetic diets) are not yet completely satisfactory replacements for diets composed of natural materials, but it is doubtful that even defined diets lack any major components. As better defined diets become available, the quality of nutritional research can be improved and the special roles of natural or processed foods can be more easily studied.

*Amino acids.* An excellent example of nutritional advances following development of highly purified diets can be seen respecting the amino acids, the need for which continues to interest many investigators. After the first task of assessing the qualitative requirements, interest has shifted to the interrelationships between amino acids and other nutrients, and among the amino acids themselves. Problems peculiar to



birds (especially the essentiality of arginine in the diet) are only part of the story. Because of the cost and limited availabilities of pure amino acids, most workers have used natural or processed proteins as the principal source of amino acids and have supplemented these with appropriate amino acids.

This approach has given, as an important by-product, information on the nutritional values of various protein sources, particularly such concentrates as the oil seed meals, fish meals and meat meals. As the amino acid deficiencies of these foods were uncovered, the possible use of synthetic or isolated amino acids has been of interest. Methionine has been available in quantity for several years, but its evaluation as a feed supplement is difficult when levels are marginal, and its costs have been high enough not to encourage widespread use in replacement of protein sources of methionine.

It is apparently not appreciated by many workers that estimation of the dietary requirement for a nutrient does not have the same biological significance as an LD<sub>50</sub>, or the level of amino acid needed to prevent change in body weight. The quantitative requirement must be based on using some arbitrary criterion for deciding at what level the requirement is met. This becomes very important when decisions have to be made about supplementation of natural diets with amino acids.

Poultry nutritionists are searching for methods of evaluating protein sources in terms of the amounts of available amino acids they contain. The need here is a very practical one for a rapid chemical or bio-assay method that will yield the amounts of each individual amino acid present in forms available to the animal. The problem is not limited to amino acids, of course, but these are particularly important in applying nutritional information to diet formulation.

*Minerals.* The sort of complex problems that are ahead of nutritionists are ex-

emplified by recent research on the minerals zinc and selenium. The dietary need for each of these is influenced very much by other components of the diet. Levels required in diets cannot be expressed without reference to the other constituents. There is a low but significant dietary requirement for zinc that, in chicks, is fairly easily satisfied by natural foods. When some purified proteins are included in the diet, however, indigestible bonds are formed with zinc, and several times the usual level of zinc must be added if an effective zinc level is to be maintained. Turkey poults are even more sensitive to this than are chicks.

Selenium, by contrast, has not been shown to be an essential nutrient, yet supplements of it are needed with certain diets containing torula yeast. Selenium plays a role in the very involved need for antioxidants such as vitamin E.

Other minerals besides zinc and selenium have been subjected to renewed scrutiny, primarily concerning their interrelationships for normal growth and egg production. Little attention has been paid to dissolved minerals (water quality) as compared to minerals supplied as feed mixtures.

*Meeting energy needs.* As the scientific approach to poultry feeding developed in the early part of the century, information from other farm animals was applied to chickens. The needs for energy were considered to be most important, and various attempts were made to evaluate feedstuffs. These were only moderately successful, however, because of intervening needs for vitamins and amino acids; while these were being studied, some work was being done on the energy metabolism of chickens and the productive or net energy of feedstuffs. The time was not right for extensive interest, however, until the late 40's, when growth and efficiency of feed utilization became important to both geneticist and nutritionist.

It was some time before the importance of dietary energy levels to protein and other nutrients was realized. Some workers felt

that good growth could be obtained only with rations high in energy; indeed, the idea that bulky feeds were valueless for poultry was widely held. Then came a series of investigations in which the diets were diluted markedly with indigestible materials such as oat hulls or wood cellulose. These studies showed that despite great dilution, birds were able to consume enough feed to maintain almost normal growth, but the body fat content was reduced. It is now generally believed that the energy level of the feed is the primary determinant of food intake, and that this in turn, greatly affects the utilization of dietary protein and other nutrients.

Attempts have been made to formalize the relationship between energy and protein as the so-called "calorie-protein ratio". In this ratio, the number of calories of productive energy per pound of diet are divided by the per cent of crude protein in the diet. The faults of this ratio have been pointed out by a number of workers, several of whom have commented on its hybrid nature and inadequacy in application. The simplest criterion to apply to figures that purport to express useful relationship is their *prediction value*. The "calorie-protein ratio" fails this test.

That there is some significant relationship between the utilizable energy and other constituents of the diet is unquestioned, especially as they are related to voluntary food intake. At present, however, an adequate measure of these interrelations has not been expressed in any simple terms.

In birds, digestion trials are complicated by simultaneous voiding of urine and feces.

This anatomical consideration and the observed variation in productive energy values of feedstuffs has led to the conclusion that, for birds, metabolizable energy is a more useful estimate of usable feed energy than is productive energy. Although much has been written concerning energy utilization by poultry, very little new information on the energy values of feedstuffs has been obtained. Only one or two laboratories in the United States have undertaken the task of providing accurate figures.

*Species studied.* Poultry nutritionists now use several birds with widely varying needs as useful tools in research. Chickens are most popular, but turkeys, ducks, geese, pheasants, quail, and pigeons have also been studied. There is increasing interest in hereditary differences in nutrient needs. A new experimental bird that shows great promise in all types of poultry research is the Japanese button quail, *Coturnix japonica*, which attains sexual maturity in less than two months and which lays eggs at a high rate. These eggs are much heavier in proportion to the hen's body weight (a 10 g. egg from a 100 g. hen) than those of chickens (a 56 g. egg from a 2000 g. hen).

One of the oldest experimental materials for biological research is the chick embryo, which still continues to be a very useful animal. Some research techniques for directly studying the nutrition of the embryo show promise of increasing the variety of nutritional problems that may be studied with chick embryos.

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## CLAY- AND CORNSTARCH-EATING WOMEN

*Clay- and cornstarch-eating pregnant and nonpregnant women from rural Alabama reveal the socio-economic, cultural and dietary factors associated with their bizarre practices.*

Pica, a craving for unnatural articles of food, and geophagy, the practice of eating

earthy substances (especially clay), have attracted the attention of curious observers

for centuries. Many hypotheses on the origin of pica and of geophagy in particular have been proposed. Some suggest that they are intuitive efforts on the part of the practitioners to compensate for nutritional deficiencies, but most regard them as cultural phenomena founded in superstition and perpetuated by tradition, especially in rural areas. There is ample evidence that pica is more prevalent among pregnant women. However, women are more influenced by cultural and social factors during pregnancy than during their nonpregnant life. It is not possible, therefore, to accept unequivocally the concept that pica is a manifestation of nutritional deficiencies resulting from parasitism or the excessive metabolic demands of pregnancy.

The ingestion of clay and cornstarch is not uncommon among women in rural areas of Alabama. Since no studies of the biochemical effects of the ingestion of clay and cornstarch have been reported, C. H. Edwards *et al.* (*J. Am. Dietet. Assn.* **35**, 810 (1959)) have initiated a series of studies designed to examine closely several aspects of this problem. The original article in this series describes the socio-economic backgrounds of the subjects and gives much information about their practices and the nutritive values of the usual diets of the clay- and cornstarch-eaters and the control subjects.

Subsequent reports will describe the relationships of clay- and cornstarch-ingestion to the results of analyses of blood and excreta of a selected sample of these women, the condition of their infants at birth and the complications during pregnancy.

The authors studied 86 women selected from prenatal and postpartum clinics. The patients were divided into six groups: pregnant and nonpregnant clay-eaters, pregnant and nonpregnant cornstarch-eaters, and, as controls, pregnant and nonpregnant women who ate neither clay nor cornstarch. Forty-two were clay-eaters, 23 were

cornstarch-eaters and six ate both. Twenty-one women served as controls. A tabulation of socio-economic data revealed that the average age was 28 years (15 to 45), 74 were married, 12 were single, they had an average of 3.8 children (0 to 11), 68 were homemakers, 13 were farmers, 5 were in other occupations, the average weekly income was \$39.00, the average weekly food budget was \$13.00, and the eighth grade was the average level for formal education (second grade to senior year in college).

Interviews revealed similarities in practices, in motivations and in emotional responses among the clay-eaters and the cornstarch-eaters. Clay was usually obtained from nearby rivers or stream banks, although it could be purchased in some areas at the roadside. Cornstarch was usually purchased. The preferred clay was light gray, although a less smooth clay, stained pink presumably by iron salts, was used by some. One brand of laundry cornstarch selling at 8 cents per box was preferred, although one woman consumed blue liquid starch. The women paid nothing for clay but spent an average of about 15 cents weekly on starch.

The clay was usually eaten uncooked, but some was baked prior to eating, especially if it was still moist. The cornstarch apparently was consumed uncooked, although specific information on this point is lacking. Clay was eaten at various times of day, sometimes with, sometimes before and sometimes after meals. Cornstarch was eaten prior to meals or, in one instance, in place of a meal. The amount of clay consumed daily ranged from 6 to 130 g. The amount of cornstarch consumed ranged from 15 g. to 2 pounds daily.

The women gave a variety of reasons for eating clay. Some had a "taste" or a "craving" for it, while others found it to have a pleasant odor or to create a pleasant sensation in the mouth. Thirty-six clay eaters felt "satisfied" after eating clay, five



claimed it relieved the nausea of pregnancy and three maintained it prevented vomiting during pregnancy. Still others affirmed its usefulness in relieving a wide variety of physical and emotional complaints.

The women gave similar reasons for eating cornstarch. "Taste", "craving", a pleasant sensation in the mouth and a pleasant odor (especially when ironing) were common explanations. Feelings of satisfaction, release of tensions and drowsiness were the desirable subjective sensations reported to follow the ingestion of starch. Morning sickness, nausea and vomiting in pregnancy were relieved in some by eating starch.

When deprived of access to their favorite, whether clay or cornstarch, both groups testified to the presence of emotional states characterized by such adjectives as "crazy", "awful", "terrible", "troubled", "disgusted", "irritable" and "worried".

No apparent physical symptoms directly associated with clay- or cornstarch-eating were reported from this study. Clay-eating had no influence on the appetite for food of 35 women, abolished the appetite for food of five and was the *sine qua non* of appetite in at least one. Surprisingly enough, cornstarch-eating had no effect on the appetite of 17 women and actually stimulated a desire for food in one woman.

Almost all the women had "cravings" or "tastes" for food only during pregnancy, rejecting the items after the births of their children.

The women studied reported knowledge of only two men who ate clay and one man who ate cornstarch, lending some credence to the theory that these forms of pica may be sex-linked habits.

A majority of the women interviewed maintained that their clay- or cornstarch-eating was of their own choice, not the result of imposed tradition and superstition. None the less, the strong forces at work in their communities to encourage pica undoubtedly

contributed at a subconscious level. Fear of harming the unborn child if clay or cornstarch were not ingested was a prime motivation among several women.

The nutritive values for the usual diets of 77 women in the study were calculated and rated according to the Recommended Dietary Allowances of the Food and Nutrition Board (*Publication 589, National Academy of Sciences-National Research Council, Washington, 1958*) as "excellent", "good", "fair" or "poor" and grouped according to the six categories of subjects. In all groups caloric intakes were below the recommended allowances and only the non-pregnant controls came close to an adequate caloric intake. In general, the diets of the control groups supplied more calories, protein, iron, thiamin, riboflavin, niacin and ascorbic acid than did the experimental groups. However all diets were low in calcium, and the diets of the pregnant clay- and cornstarch-eaters contained substantially less than recommended amounts of iron, thiamin and niacin. Fifty-four per cent of the diets of clay-eaters and 33 per cent of the diets of cornstarch-eaters were classified as "poor", whereas only 14 per cent of those of the controls were so rated. The authors suggest that the self-selected diets of the clay- and cornstarch-eaters may have precipitated a need for additional nutrients which may have been supplied by clay (iron and calcium) and cornstarch (calories).

Although the interpretation of these findings as indicating nutrient deficiencies may be premature, the subsequent articles dealing with medical and biochemical data from these patients should be of interest. Additional well-controlled studies of the influence of dietary supplementation on the prevalence of pica would lend considerable support to the theory of a nutritional etiology for this bizarre habit.

The authors have, in addition to presenting their own findings, reviewed a fascinat-



ing literature on the general subject of pica. The work of M. Cooper (*Pica: A Survey of the Historical Literature as Well as Reports from the Fields of Veterinary Medicine and Anthropology, the Present Study of Pica in*

*Young Children, and a Discussion of Its Pediatric and Psychological Implications.* Charles C. Thomas, 1957) contains historical details of pica from ancient to modern times.

## HYPOGLYCEMIA INDUCED BY GALACTOSE

*Pronounced elevation in blood galactose may be associated with hypoglycemia.*

Galactosemia, an hereditary disorder which appears in infants, is evidenced by an increased galactose level in the blood (as shown by increased total reducing sugar levels) and by poor growth. The metabolic effects of this hereditary disorder have been reviewed earlier (*Nutrition Reviews* 14, 5 (1956)). Because of an accumulation of galactose-1-phosphate, it is believed that galactosemia is due to a deficiency of a specific enzyme (galactose-1-phosphate uridyl transferase) which allows further metabolism of this compound (*Ibid.* 17, 115 (1959)).

Recently, T. L. Dormandy, D. Leak and M. Grant (*Lancet* II, 269 (1959)) have studied the effect of galactose on the level of glucose in the blood. Using the glucose oxidase method for the determination of blood sugar, it was possible for these investigators to determine changes in the glucose content of the blood when there were large quantities of galactose or other reducing sugars present.

In studying six volunteers, each of whom was given an oral galactose tolerance test after taking 50 g. of galactose, transient slight falls in blood glucose were noted in only three of the six individuals. After receiving 100 g. of galactose by mouth, four of the six volunteers showed only a slight decrease in blood glucose during the first hour following the ingestion of the sugar. However, when a high blood galactose level was obtained by giving the galactose intravenously, five of the six volunteers showed depressions in the blood glucose levels within a few minutes after the ga-

lactose had reached its peak value (total reducing sugar about 325 mg. per cent). Under these conditions the maximal decrease in the blood glucose amounted to approximately 30 mg. per cent and was persistent for about 90 minutes.

Thus it appears that with this group of volunteers (apparently adults) the elevation of the total blood sugar produced by giving galactose intravenously caused a fall in the blood glucose levels.

Since lactose (hydrolyzed to galactose) may be less efficiently utilized in the neonatal period than it is in the adult, these investigators carried out preliminary studies on six prematures and three full-term infants. They did not believe that galactose tolerance tests, either oral or intravenous, need be carried out on these newborn babies for this series of studies. However, they studied the galactose and glucose levels while the infants were maintained on their ordinary feeding schedules, and in this manner employed a "rough and ready" galactose and glucose tolerance test. In the case of seven of the nine infants, there was an increase in the blood glucose levels in the hour and a half following the feedings.

However, in one premature infant and one premature and jaundiced infant there were marked decreases in the blood glucose along with the elevation of the total blood sugar during the early period following the oral feeding. On the second day of life for the first infant, the fasting blood sugar was 47 mg. per cent and it fell to a minimum value of 15 mg. per cent 45 minutes after the feeding. By the third day of life the

tolerance of galactose had increased so that, in spite of a minimum blood glucose value of 19 mg. per cent reached 15 minutes after feeding, the blood glucose returned to the fasting level within one hour after feeding, remaining at approximately the same level for the next hour.

The tests were repeated on the tenth and twentieth days and, in both cases, the blood glucose increased greatly after feeding with only small increases in the total reducing sugar levels.

Similar trends were noted in the second premature infant who was jaundiced, and in this case, while the fasting blood glucose was 32 mg. per cent on the third day of life, it fell to a minimum value of 13 mg. per cent 30 minutes after feeding and attained a fasting value about 30 minutes later. During this time, the total blood sugar (representing mainly galactose) steadily increased, reaching a maximum value at 30 minutes (90 mg. per cent). On the fourth day of life, similar levels of blood glucose were noted, but by the ninth day there was an increase in the blood

glucose level after ingesting food and the galactose was at its maximum, only 20 mg. per cent.

While none of these individuals showed definite clinical signs of hypoglycemia, the possibility could not be excluded that hypoglycemia produced by galactose could be a clinical as well as a biochemical feature in the neonatal period. The chemical detection of such a hypoglycemia would require the use of the glucose oxidase method.

The means by which the increased blood galactose influences the blood glucose is not known. The possibility exists that the blood galactose stimulates the pancreas directly to produce increased amounts of insulin. Also, the release of glucose by the liver may be influenced by the elevated blood galactose. These investigators suggest that the metabolic regulatory system responsible for maintenance of the blood glucose within its normal narrow limits apparently cannot readily differentiate between glucose and galactose. Thus a sharp rise in the blood galactose will cause a decrease in the blood glucose level.

## VANADIUM INHIBITION OF CHOLESTEROL SYNTHESIS IN MAN

*Vanadium inhibition of cholesterol synthesis in man resulted in lowering of serum cholesterol. Balance studies indicated that tissue cholesterol stores were also reduced.*

Treatment of atherosclerosis has been primarily concerned with reduction of serum cholesterol. This has followed the demonstration that a significant correlation exists between coronary atherosclerosis and elevated serum cholesterol (D. Davis, B. Stern and G. Lesnick, *Ann. Int. Med.* 11, 354 (1937)).

There are at least three ways by which body cholesterol may be lowered. First, the degradation of cholesterol to bile acids may be increased, but there is apparently no non-toxic way of doing this at present. Also, primary absorption of dietary cholesterol or reabsorption of biliary cholesterol can be diminished. The result is a readjust-

ment of synthesis by the liver to compensate for the decrease in cholesterol returned to the circulation. Thus it appears that decreasing the synthesis of cholesterol is the best method for controlling the cholesterol content of the body.

One method of reducing synthesis is by decreasing the intake of fat. Also, the ingestion of increased amounts of unsaturated fatty acids will reduce synthesis. In view of the difficulty encountered in trying to change food habits, the use of an inhibitor which can interfere with some step in the intermediary synthesis of cholesterol may prove to be advantageous. G. L. Curran and D. L. Azarnoff (*Arch. Int. Med.* 101,

685 (1958)) have discussed the properties of an ideal inhibitor. According to their reasoning, inhibition should precede the step at which squalene cyclizes to form the steroid nucleus. Sterols other than cholesterol have been found in atherosclerotic plaques. On the other hand, blocking the synthesis of acetyl-coenzyme A from acetate would have little influence on cholesterol synthesis since acetoacetate, which follows in the sequence, is also derived from fat catabolism.

Salts of vanadium have the unique property of inhibiting the utilization of mevalonic acid for cholesterol synthesis according to Azarnoff and Curran (*J. Am. Chem. Soc.* **79**, 2968 (1957)). Curran and R. L. Costello observed that deposition of cholesterol in rabbit aorta was inhibited by vanadium salts (*J. Exp. Med.* **103**, 49 (1956)). In a recent review (*Nutrition Reviews* **17**, 231 (1959)) it was disclosed that workers subjected to vanadium fumes or dust show a significantly lower serum cholesterol when compared with fellow workers not so exposed.

Curran, Azarnoff and R. E. Bolinger (*J. Clin. Invest.* **38**, 1251 (1959)) have recently examined the effects of vanadium on sterol balance in normal men on a rigidly controlled diet.

The subjects were five normal male medical students, ages 23 to 26. All were fed identical portions of a diet which was constant throughout the study. After a two-week control period, 100 or 125 mg. of diammonium oxy-tartratrovanadate were given daily for a six-week experimental period. Finally, there was a three-week recovery period without vanadium. Serum cholesterol, phospholipid, triglyceride, alkaline phosphatase and transaminase were determined. Urine analysis for vanadium, 17-ketosteroids, and 17,21-dihydroxy-20-ketosteroids was done at the beginning and end of the vanadium administration period. Three-day stool collections were frozen,

lyophilized, ground and stored under vacuum prior to analysis.

During the administration of vanadium the level of serum free and total cholesterol fell significantly, while during the subsequent recovery period it rose once more. Vanadium did not appear to influence serum phospholipids, and the cholesterol to phospholipid phosphorus ratio was significantly lowered by the eighth week but was again elevated by the end of the recovery period.

Serum triglycerides rose significantly during vanadium administration and then fell after treatment was discontinued. Fecal fat did not change, indicating that vanadium did not interfere with absorption of fat from the gastrointestinal tract. Total fecal sterol excretion decreased during vanadium administration but increased again during the period without vanadium.

Vanadium balance studies show that about 60 per cent of ingested vanadium is excreted in urine in a 24-hour period, according to studies made by N. A. Talvitie and W. D. Wagner (*Arch. Indust. Hyg.* **9**, 414 (1954)). Determinations of urinary vanadium in the report of Curran, Azarnoff and Bolinger revealed that vanadium absorption was variable from individual to individual and from time to time. At the end of vanadium administration analyses of urinary vanadium were continued along with determinations of serum free cholesterol. The per cent lowering of serum free cholesterol remained at about 20 per cent while urinary vanadium excretion fell from 80 to 20 micrograms per day. Subsequently, the lowering of serum cholesterol decreased to 13 and then to 8 per cent as vanadium excretion dropped to 15 and then 10 micrograms.

If the vanadium excretion is calculated as micrograms per pound of body weight, then a maximal lowering of free cholesterol in the serum appears to be maintained while vanadium excretion is as low as 1 microgram per pound of body weight per day.

There was no obvious evidence of toxicity from vanadium, and no changes were observed in complete blood counts, routine urinalyses, blood urea nitrogen and blood glucose during vanadium administration. Evaluation of liver function by analysis of cholesterol esters, serum alkaline phosphatase, serum transaminase and serum bilirubin showed no change. Analysis of urinary 17-ketosteroid and 17,21-dihydroxy-20-ketosteroid excretion showed no inhibition by vanadium.

The 20 per cent lowering of serum cholesterol was the maximum effect produced. However, Curran, Azarnoff and Bolinger point out that the subjects had normal cholesterol levels, and that the rate of cholesterol synthesis and the lipoprotein transport system were probably normal. In order to determine whether higher levels of serum cholesterol might be further reduced by this treatment, a clinical trial of vanadium in hypercholesterolemic individuals would be necessary.

Although this study presents no direct evidence that endogenous cholesterol synthesis has been inhibited, findings in experimental animals have indicated that this is likely. In addition, *in vitro* studies by Azarnoff, Curran and W. P. Williamson (*J. Nat. Cancer Inst.* **21**, 1109 (1958)) have demonstrated that vanadium inhibits the incorporation of acetate-1-C<sup>14</sup> into cholesterol in human intracranial tumors.

The reduction in fecal sterol output may be explained in one of three ways. The reduction could be due to an increase in retention of cholesterol in tissues with no change in endogenous synthesis. It also could be the result of a lowering of synthesis with no net change in tissue cholesterol content, or it may represent decreased synthesis and diminished tissue cholesterol stores as well as reduced output of fecal sterol.

The first possibility is unlikely in view of the experimental evidence that cholesterol synthesis is inhibited and tissue storage of

cholesterol is decreased in rabbits after vanadium administration (J. T. Mountain, F. R. Stockell, Jr., and H. E. Stokinger, *Proc. Soc. Exp. Biol. Med.* **92**, 582 (1956)). Analysis of livers of two patients with brain tumors who had received vanadium for four and eight months respectively, showed cholesterol levels of 225 and 250 mg. per 100 g. of liver. These values are below the average normal of 300 mg. per 100 g. for a man weighing 70 kg.

In order to determine whether inhibition of cholesterol synthesis has resulted in decreased tissue stores, Curran, Azarnoff and Bolinger determined sterol balances in their experimental subjects. Dietary sterol, fecal neutral sterol and fecal bile acids were determined. Urinary sterols were assumed to be negligible, serum sterols constant, and conversion to steroid hormones unaffected. By incubating feces with and without added vanadium it was demonstrated that the vanadium did not produce its effect by increasing bacterial destruction of sterol. The amount of dietary sterol absorbed was determined by comparing the control period with the experimental periods.

Dietary sterol following ingestion is partly absorbed and mingles with synthesized sterol to make up the total body sterol, which equals the sum of tissue and serum sterols. About 25 per cent of absorbed dietary sterol is excreted along with bile acids and endogenous neutral sterols. This total excreted sterol, plus unabsorbed dietary cholesterol, comprises the output from the intestines. The authors grant that the amount of absorption and excretion of dietary cholesterol could be more accurately determined if isotope techniques were possible for man. Nevertheless, assuming the basis for calculation to be sound, they believe that the trends are correct for body and tissue sterol changes.

In order to determine whether vanadium inhibition reduces tissue cholesterol levels, its synthesis during the recovery period

was calculated. The change in body sterol was assumed to be zero.

To calculate the synthesis during vanadium treatment, the ratio of excreted sterol during vanadium administration to excreted sterol during recovery was considered proportional to the ratio of synthesis values in the two periods. This assumption was based on the observation that synthesis was the major contributor to excreted sterol as well as on the fact that the calculation of the extent of reduction in synthesis gave values less than would be expected from previously mentioned isotope studies of synthesis depression in animals and humans. Since the period of greatest change, namely from the control period to the vanadium period, was not used in the calculation of synthesis inhibition, it may be assumed that

the calculated values are conservative minimums.

In spite of minimizing factors, a reduction in body and tissue sterol content occurred during the vanadium period. One man showed a large reduction in total serum cholesterol while tissue sterol was increasing, showing the importance of calculating tissue sterol content.

Although the details of the calculations cannot be given here, it is evident that a great deal can be learned by careful balance studies. This method should be valuable in comparing the effects of several cholesterol lowering compounds now under active investigation. Synergistic effects should be sought between vanadium and compounds decreasing dietary cholesterol absorption.

### URIC ACID DEGRADATION IN MAN

*Extrarenal elimination of uric acid in man via the gastrointestinal tract is stressed. Human tissues do not have the enzyme systems to degrade uric acid, but this can be done by the intestinal flora.*

For the complete degradation of uric acid, one requires enzyme systems consisting of uricase, allantoinase, allantoicase, and urease. Thus, in the stepwise breakdown of uric acid, the following compounds are formed in sequence: allantoin, allantoic acid, urea, glyoxylic acid, and finally carbon dioxide and water. Depending upon which of the various enzymes are missing in different species, purine metabolism may end with the formation of uric acid, allantoin, allantoic acid, urea or ammonia, and carbon dioxide.

The metabolism of uric acid in man has been the subject of discussion for many years. Earlier investigators reported values ranging from almost complete recovery to 28 per cent of the injected uric acid appearing in the urine, the usual average being about 50 per cent. After the induction of isotope techniques, it has been reported that between two thirds and three fourths

of the isotope injected in man as uric acid can be recovered as urinary uric acid.

Recently L. B. Sorensen (*Metabolism* 8, 687 (1959)) has reported studies involving the degradation of uric acid in man. Uric acid labeled in the 2 position with  $C^{14}$  was injected intravenously into five individuals who had been maintained on a purine free diet for five days before the study. This group consisted of two normal individuals, two gouty subjects and one patient with arteriosclerotic heart disease. The urine was collected from these patients for ten to 13 days following the administration of the tagged uric acid. At the end of this time the  $C^{14}$  content of the body uric acid had fallen to within a few per cent of the concentration at zero time.

Sorensen found that the intravenously injected tagged uric acid was not recovered quantitatively as urinary uric acid. In the two normal individuals recoveries of 64.3

and 68.9 per cent of the administered uric acid were obtained from the urine, while recoveries of only 46 and 46.3 per cent of the administered material were obtained in the urine of the two gouty subjects. As expected, the miscible uric acid pool in the gouty individuals was almost twice that of the normals, although both patients had only a short history of gout.

It appeared, therefore, that the mean daily urinary excretion of uric acid was less than the amount of uric acid being replaced per day by the body. For the two normal subjects the amount of uric acid in the urine was only 57.9 and 69.5 per cent of the calculated turnover of uric acid, while the values for the patients with gout were 47.8 and 45.8 per cent. From these calculations it appeared that a surplus of 262 or 213 mg. of uric acid was being formed over the amount excreted in the urine by the normal individuals, while the gouty patients had surpluses of about twice these values. On the other hand, the value for the patient with arteriosclerotic heart disease was within the normal range. Thus these observations indicated that there must be some pathway of excretion of uric acid other than a urinary route.

Since some  $C^{14}$  was found to be incorporated into allantoin, allantoic acid, urea and carbon dioxide, following the intravenous administration of uric acid, one has to assume that some breakdown of uric acid takes place in the human body. In the normal subjects it was found that 2.1 and 3.9 per cent of the injected uric acid  $C^{14}$  was recovered in urinary allantoin two and five days respectively after administration. However, in the case of the two gouty patients, 4.7 and 0.4 per cent of the isotope appeared in allantoin five and two days respectively after the tag was given.

While Sorensen did not quantitatively determine the allantoin in the urine, he estimated the amount of urinary allantoin resulting from the degradation of uric acid. It was calculated that an average of 24 mg.

of urinary allantoin had been derived daily from uric acid in the four patients. Upon comparing this value to the reported average daily output of allantoin in the urine (10 to 100 mg.), one might assume that a considerable amount of urinary allantoin is derived from the actual breakdown of uric acid in the body.

After the administration of uric acid tagged with radioactive carbon,  $C^{14}$  could be noted in the allantoic acid and urea fractions of the urine. It was calculated that about 2.6 per cent of the uric acid  $C^{14}$  appeared in the urinary allantoic acid during the first 12 hours after the isotope had been given. In all five patients significant amounts of  $C^{14}$  were found in the urea. In the case of two normal individuals, 1.7 and 2.2 per cent of the injected uric acid  $C^{14}$  was found in the urinary urea 13 and 15 days, respectively, after the administration of the tagged uric acid. Similar values were found in two patients with arteriosclerotic heart disease (one new patient and the one described earlier), as well as in a gouty patient.

Should the  $C^{14}$  tagged uric acid be degraded to such an extent that carbon dioxide is formed in the body, then there should be some loss of activity in the expired air. The two normal persons excreted 4 and 11 per cent of the injected  $C^{14}$  through the lungs one and four days, respectively, after the administration of the tagged uric acid. Seven per cent of the dose was recovered in the air expired by a gouty patient over a 48-hour period.

Thus in these studies it was observed that administered uric acid is degraded in the human body and that the radioactivity in an initial dose of  $C^{14}$  labeled uric acid will appear in allantoin, allantoic acid, urea, and carbon dioxide. When  $C^{14}$  labeled uric acid was given to patients by intravenous administration, the renal excretion of uric acid amounted to only 50 to 70 per cent of the administered dose. Hence the question arises as to whether the material is ex-

creted in the digestive juices and whether there is degradation of uric acid in the intestinal tract.

Because of the lack of specific methods, Sorenson did not accept as reliable earlier reports suggesting that between 40 and 70 mg. of uric acid were excreted in the digestive juices per day and that 10 to 20 mg. of this may occur in the feces. Using the method of differential spectrophotometry, it was found that 3.1 (1.7 to 4.6) mg. per cent of uric acid occurred in the saliva of ten normal men. The average value in the saliva of 12 normal women was lower, 1.9 (0.9 to 2.8) mg. per cent. The daily excretion of uric acid in the saliva was estimated as between 30 and 50 mg. per day for men.

On two occasions it was found that saliva from a gouty subject contained 6.67 and 6.24 mg. per cent of uric acid, a value which is considerably above the normal limit. If this patient produced 1.5 liters of saliva per day, then he lost no less than 100 mg. of uric acid per day in the saliva, making it one of the major pathways of uric acid excretion.

Gastric juice was found to contain 0.5 to 1.0 mg. per cent of uric acid. Uric acid assays on sterile bile obtained from the fistulae of ten patients, in whom a choledochotomy was performed in connection with a cholecystectomy, yielded an average uric acid concentration of 4.4 mg. per cent. Thus, if a patient were producing one liter of bile per day, he would lose about 50 mg. of uric acid in this secretion.

Sorensen believes that the daily excretion of uric acid in the saliva, gastric juice and bile in normal man amounts to about 100 mg. per day, and somewhat less in women. It was postulated that if similar amounts of uric acid are expected in pancreatic and intestinal juice, then at least 200 mg. per day are lost from the body through the intestinal route. Thus, he believes that approximately one-third of the uric acid formed is disposed of through the digestive juices in normal

individuals, and in the case of patients with hyperuricemia, the extrarenal elimination plays a more important role.

Sorensen also believes that the rate of uricolysis in the intestinal tract, as judged from these studies, is sufficient to account for the degradation of one third of the uric acid turned over in normal human beings.

Since uric acid is poured into the intestine by the various digestive juices, the question arose as to the role of intestinal flora in uricolysis in man. This problem was studied by determining the rate of turnover, the miscible pool size, and the recovery of  $C^{14}$  uric acid metabolic products both before and after effective bacteriostasis was induced in the intestine by the administration of antibiotics and sulfa drugs.

The miscible pools in the two normal subjects were approximately the same and the milligrams of uric acid turned over per day (699 and 710 mg.) were similar, along with urinary excretion (480 and 415 mg.) both before and after effective bacteriostasis was established. Sixty-nine per cent of the administered  $C^{14}$  was recovered as urine uric acid initially, and after the intestine was sterilized the value dropped to 55.7 per cent. During the control period 7.2 per cent of the injected dose of radioactivity was found in the feces, but since no uric acid could be demonstrated, it was assumed that the activity was due to  $C^{14}$  incorporated in the bacteria and degradation products of uric acid.

After the bacteriostasis had been well established uric acid was constantly found in the feces, and a total of 930 mg. was excreted over a five day period following the injection of the purine. This corresponds to the 26.6 per cent of the total uric acid turnover, while at the same time 30 per cent of the amount of the injected activity was recovered in the stool, indicating that essentially all the  $C^{14}$  in the feces was incorporated in uric acid.

Before antibiotics were given to the



patient who received the uric acid intravenously, 2.1, 0.2, 2.2 and 10.9 per cent of the administered dose of radioactivity was found in the urinary allantoin, allantoic acid, urea, and in the carbon dioxide expired in the air, respectively. Following the sterilization of the intestine, however, only 1.8, essentially none, 0.7 and 0.5 per cent of radioactivity was found in the corresponding urinary fractions. Before sterilization 7.1 per cent of the dose of uric acid  $C^{14}$  was found in the stool as degradation products, while after sterilization essentially none was found. Thus, with an active intestinal flora, 22.5 per cent of the uric acid  $C^{14}$  was recovered in different metabolic degradation products. This dropped to 3 per cent after a high degree of intestinal bacteriostasis was established. The author suggests that the intestinal flora has an important role in the degradation of uric acid in man.

Next, Sorensen studied whether or not there was uricolytic activity in human tissue, *i.e.*, processes which will break down uric acid to form simpler products. To study this, he used uric acid tagged in the C-6

position with radioactive carbon. Samples of liver, kidney, spleen, pancreas and whole blood (all obtained at autopsies) were tested to see whether or not, upon incubation of the homogenate of the tissues with the tagged uric acid, there would be an increased radioactivity in the carbon dioxide produced. Sixty-four different experiments were carried out with different human tissues and the amount of carbon dioxide liberated in all cases was negligible. Thus it would appear that uric acid is not broken down in the human tissues tested, possibly indicating that the enzyme systems responsible for the breakdown of uric acid do not exist in these tissues.

This work may be summarized as follows. Uric acid is not enzymatically degraded in human tissues. However, a considerable amount of uric acid is excreted into the gastrointestinal tract where it may be broken down by the bacteria present in the flora. Extrarenal excretion eliminates a considerable portion of the uric acid produced each day.

### VITAMIN $B_{12}$ AND GROWTH OF CHILDREN

*Oral administration of 20 micrograms of vitamin  $B_{12}$  daily to underdeveloped children in Central America failed to alter their rate of increase in height or weight.*

Since 1949, when vitamin  $B_{12}$  therapy was reported to stimulate the growth of children (N. C. Wetzel *et al.*, *Science* **110**, 65 (1949); *Nutrition Reviews* **8**, 139 (1950)) there has been continuing interest in the possibility that vitamin  $B_{12}$  deficiency might be a fairly general cause of growth failure (*Nutrition Reviews* **10**, 146 (1952); **11**, 42 (1953)). During the past ten years there have been many attempts to test this possibility and the published reports of these tests have been reviewed by E. E. Howe (*Am. J. Clin. Nutrition* **6**, 18 (1958)). The results taken all together are inconclusive, since both negative and positive results were obtained.

A further study of the effect of oral administration of 20 micrograms of vitamin  $B_{12}$  daily to Guatemalan children has been reported by N. S. Scrimshaw, J. A. Muñoz, O. B. Tandon and M. A. Guzmán (*Am. J. Clin. Nutrition* **7**, 180 (1959)). Their experiment merits attention since it was particularly well controlled and was carried out on children having an extremely low intake of animal protein, whose development was from two to four years behind that of well-nourished children of comparable age.

The experimental subjects were 278 school and preschool children in five small rural villages. They were ranked according

to age and sex and were assigned randomly to placebo or experimental groups. Their daily intakes of animal protein ranged from 8 to 14 g. They ate ad libitum in their own homes throughout the study and, six days a week, were given orally tablets containing 20 micrograms of vitamin B<sub>12</sub> or a placebo tablet identical in appearance. Even the personnel administering the tablets did not know which children were receiving vitamin B<sub>12</sub>.

Height and weight measurements were taken monthly for one year on a group of 50 of the preschool children, and for 18 months on four groups (228 individuals) of the school children, except for twelve weeks when they were on vacation. The height and weight gains were adjusted for initial differences in age, height and weight by an analysis of covariance.

The results obtained in the experiment with preschool children gave no indication that vitamin B<sub>12</sub> administration had influenced the rate of increase in either height or weight. The results on the four groups of school children were calculated for three successive six-month periods to permit twelve comparisons. In ten of these no effect of vitamin B<sub>12</sub> was discernible; in one comparison a significant positive effect was obtained and in another a significant negative effect was obtained. In one trial in the experiment on school children the average increases in height, with and without vitamin B<sub>12</sub>, were 0.37 and 0.36 cm. per month, respectively, and gains in weight of 0.20 and 0.18 kg. per month, respectively.

The authors conclude from these results and from a survey of the available literature on the subject, that vitamin B<sub>12</sub> supplementation is without effect on the nutritional status of children from technically underdeveloped areas whose growth and development is retarded.

They discuss some of the problems in studies of this type, emphasizing the need for extreme care in selecting the most reliable control. Differences in the growth

rates of children in the different villages make it clear that almost any type of result could be obtained by using children of one village as the control group and those from another as the experimental group. Also, large differences in the growth rate of a single group of children from one six-month period to another precluded comparisons between successive periods (with and without supplementation) where each group served as its own control.

Moreover, the authors emphasize the fallacy of reporting beneficial effects of supplementation on a few individuals in an experimental group who show unexpectedly large responses, since, in their study, such individuals were found with equal frequency in both experimental and control groups. Some of these problems have been discussed previously (*Nutrition Reviews* 16, 287 (1958)).

The main criticism that can be made of experiments of this type on underdeveloped children is that raised by Howe (*loc. cit.*), i.e., would these children have responded to the treatment if some other dietary factor had not been limiting the response? Their experiment does not answer this criticism but Scrimshaw *et al.* cite a previous study (Scrimshaw and M. A. Guzman, *Nutrition Symposium Series No. 7*, p. 101 (1953); *Nutrition Reviews* 12, 271 (1954)) in which dietary supplements were included with vitamin B<sub>12</sub> to make up for existing deficiencies and in which, again, no beneficial effect of vitamin B<sub>12</sub> could be demonstrated.

These observations offer a challenge to those who believe that vitamin B<sub>12</sub> administration may produce a growth response in well-nourished children whose growth and development is retarded for their age. The Committee on Nutrition of the American Academy of Pediatrics attempted to resolve the question by undertaking several critical and carefully controlled experiments with children, but no convincing evidence was found that vitamin B<sub>12</sub> promotes growth (*Nutrition Reviews* 16, 287 (1958)).

## PHENYLACETIC ACID AND THE PRODUCTION OF SEROTONIN

*The increased production of 5-hydroxytryptamine (serotonin) could be reduced in certain individuals by feeding phenylacetic acid, believed to act as an inhibitor of 5-hydroxytryptophan decarboxylase.*

The role of serotonin (5-hydroxytryptamine) in various biological reactions has been reviewed earlier (*Nutrition Reviews* 15, 156 (1957); 16, 85 (1958)). In certain carcinoid tumors of the large bowel, excessive amounts of serotonin are being produced which get into the general circulation and are responsible for many of the symptoms such as diarrhea and flushing which occur in these patients. It is believed that serotonin is produced biologically from 5-hydroxytryptophan. A. A. Davison and M. Sandler (*Nature* 181, 186 (1958)) have shown that some of the aromatic acid metabolites of phenylalanine inhibit *in vitro* the decarboxylation of 5-hydroxytryptophan, thus decreasing the rate of formation of 5-hydroxytryptamine.

With this in mind, Sandler and H. G. Close (*Lancet* II, 316 (1959)) studied the effect of administering phenylacetic acid to a patient who had carcinoid tumors which were secreting 5-hydroxytryptophan. Five grams of phenylacetic acid were given to this patient after obtaining a one hour urine collection, and urines were then collected at periods of one hour for the next 11 hours. Under similar conditions, a patient was studied who did not receive the phenylacetic acid.

All the urine samples were analyzed to determine the amount of 5-hydroxyindoleacetic acid and 5-hydroxytryptamine excreted per hour. 5-Hydroxyindoleacetic acid was determined since it is believed to be the index of serotonin metabolism. A decrease in both the 5-hydroxytryptamine and the 5-hydroxyindoleacetic acid was noted after the patient received the phenylacetic acid, although the decrease in the indoleacetic acid was greater than that of the serotonin. This would indicate that

there was an *in vivo* inhibition of 5-hydroxytryptophan decarboxylase.

As well as analyzing the effects of a single dose, these investigators gave the patient 6 g. phenylacetic acid per day for a period of five successive days. Since actual values for the urinary excretion of serotonin or 5-hydroxyindoleacetic acid are not given for this period, one must assume that there was no inhibition of the production of serotonin when more than a single dose of phenylacetic acid was given.

In the second study, M. Sandler, A. Davies and C. Rimington (*Lancet* II, 318 (1959)) observed the effect of administering 5 g. of phenylacetic acid on the urinary output of 5-hydroxyindoleacetic acid in five patients with the carcinoid syndrome during an 11-hour period following the administration of the acid. In three of the five patients there was a marked decrease in the levels of 5-hydroxyindoleacetic acid found in the urine, caused, the authors believe, by the inhibiting effect of phenylacetic acid on 5-hydroxytryptophan decarboxylase, an enzyme capable of producing serotonin from 5-hydroxytryptophan.

To study the effect of daily doses, two of the individuals with carcinoid tumors received 6 g. of phenylacetic acid per day for six successive days. However, the output of 5-hydroxyindoleacetic acid was the same as during the control period. One of these two, however, had a marked response to the inhibitor on the 11-hour single dose test while the other had no response. Also, three patients who had received phenylacetic acid in a double blind test for 14 successive days exhibited no significant difference in the flushing or diarrhea when compared with the placebo periods.

The question arose as to why the phenylacetic acid was an effective inhibitor of the

production of serotonin when studied over a short period (11 hours) but had no effect when given over a longer period. While at present there are a few suggested explanations, there is little evidence to prefer one over the other. In explaining the varied ability of phenylacetic acid to inhibit the production of serotonin in different patients, the authors suggested that perhaps there may be differences in the ability of the phenylacetic acid to penetrate the tumor mass sufficiently rapidly to allow for the

accumulation of an effective inhibition level.

However, it should be noted that M. D. Milne (*Lancet* **II**, 467 (1959)) questions whether the phenylacetic acid inhibits the 5-hydroxytryptophan decarboxylase *in vitro*, or whether there is a temporary blocking of the renal excretion of the 5-hydroxyindoleacetic acid.

At present this problem is not solved, but it is unlikely that it will be without a solution for long.

### VOLATILE FATTY ACIDS FOR CORRECTION OF LOW FAT MILK

*Volatile fatty acids fed to cows producing low fat milk as a result of low roughage diets at least partially restore the butterfat level of the milk.*

The well-established observation that diets low in roughage and high in concentrates will usually result in the production of low butterfat milk by dairy cattle has aroused considerable interest because of the bearing this has on both normal ruminant and non-ruminant fat metabolism. The ruminant differs from the non-ruminant in the large quantities of fatty acids absorbed directly from the rumen-reticulum. These fatty acids are metabolized as a source of energy and, in dairy cattle, are converted into butterfat to a considerable extent.

The marked disturbance in fatty acid synthesis occurring in the rumen-reticulum with high-concentrate low-roughage diets has led to experiments in which dairy cattle have been fed the volatile fatty acids (sodium acetate, sodium propionate, and sodium butyrate) in an effort to maintain the fatty acids in the rumen-reticulum at normal levels and proportions and achieve a normal level of butterfat synthesis.

The study of milk of low fat content at Reading, England, has been recently extended by C. C. Balch and S. J. Rowland (*J. Dairy Res.* **26**, 162 (1959)). They studied the action of sodium acetate, sodium propionate and sodium butyrate in animals fed a normal diet producing a normal level of

butterfat as well as in Shorthorn cattle fed diets resulting in milk with a low fat content. Cattle fed a low hay diet for three weeks showed a significant decrease in the fat content of the milk, and the butterfat was further reduced with continued feeding of this diet. In most animals the butterfat content leveled out at a low percentage after 11 weeks on the diet.

These investigators determined the Reichert number for the fat in the milk and found good evidence for a relationship between fatty acid synthesis and the composition of butterfat. Acetate is absorbed directly from the reticulo-rumen and has been shown to be the source of part of the milk fat (G. Popják, T. H. French and S. J. Folley, *Biochem. J.* **48**, 411 (1951)), and to be particularly active in the formation of fatty acids containing up to 16 carbon atoms. With lowered fat production in the milk there was a decrease in the Reichert number, while with the return to a normal butterfat content, the Reichert number increased, especially when sodium acetate was added to the diet.

Perhaps one of the most interesting aspects of this work by Balch and Rowland is the indication that acetate and butyrate may be important, but perhaps not the

controlling factors, in butterfat synthesis. When they added from 0.5 to 1.5 kg. of sodium acetate to the diet of cows in which the fat content of the milk had dropped from 4.8 per cent to a low of 2.8 per cent, there was a rapid (although not complete) recovery of the butterfat level. When sodium propionate was added there was no recovery. However, in one instance when sodium butyrate was added recovery was almost as good as with sodium acetate.

Although these investigators aimed at achieving adequate levels of acetate in the rumen-reticulum, the response to acetate indicated that this is only part of the mechanism required for normal butterfat production in dairy cattle. Further indication of this was the observation that the addition of fatty acids as sodium acetate, propionate or butyrate to normal roughage diets of cattle producing milk with a normal fat content had no effect upon either the fat content or the Reichert number of the milk.

Balch and Rowland also investigated the possibility that very succulent silage causes a reduction in butterfat content because of lack of fiber. Their results differed somewhat from those of Z. Zelter (*C. R. Acad. Sci. Paris* **231**, 1574 (1950)) who suggested that the fibrousness of the silage may be a more controlling factor than the presence or absence of fatty acids in the silage.

While the work of Balch and Rowland was carried out on a comparatively small number of animals, their well-controlled investigation emphasizes the importance of the synthesis of fatty acids in the rumen in the production of milk as well as non-fatty solids. More importantly, however, their work emphasizes the fact that this is only a part of the overall metabolic picture respecting butterfat production. Further knowledge is needed concerning the mechanism whereby fiber in the diet results in normal fatty acid production, the importance of the proportion of different fatty acids, and other factors controlling butterfat synthesis.

## DAIRY PRODUCTS AND DENTAL CARIES

*Substitution of part of a cariogenic diet of weanling rats by various dairy products reduces tooth decay. Similar alterations of the maternal diet are without influence.*

The influence of dietary carbohydrate in increasing the susceptibility of human subjects to dental caries was clearly shown by the Vipeholm study (*Nutrition Reviews* **12**, 233 (1954)). Such a study, however, is laborious, expensive and time-consuming, and the development of a strain of caries-susceptible rats has aided materially in the study of the caries-initiating influence of various diets and foodstuffs, at least in a preliminary way (J. H. Shaw, *J. Nutrition* **41**, 13 (1950)). It has been found, for example, that rats raised on a milk diet are resistant to the cariogenic influence of dissolved sugar but not dry sugar (*Nutrition Reviews* **13**, 316 (1955)). It was not known, however, whether this influence of

milk extended to the developmental period or was uniquely concerned with the post-eruptive oral development.

In a study designed to clarify this point, J. H. Shaw, B. J. Ensfield and D. H. Wollman (*J. Nutrition* **67**, 253 (1959)) carried out two experiments in which dairy products were substituted for a part of the cariogenic diet of either or both the maternal or filial generation of susceptible rats.

In the first experiment, 38 female rats were divided at weaning into four groups which were fed either the cariogenic diet alone or supplemented with whole milk, chocolate milk or chocolate drink (in which all but 2 per cent of the milk fat was removed) to provide, respectively, 30, 30 or

35 per cent of the daily caloric intake. After 24 weeks, they were bred to caries-susceptible males and the young of each group were maintained from weaning to 63 days on either the cariogenic diet or one of the dairy products, and from 63 to 121 days on the cariogenic diet alone or supplemented with one of the dairy products providing 33 per cent of the day's calories.

All animals in the maternal generation, although on different diets from the time of weaning, had similar growth and other gross characteristics. However, all those with the dairy supplements had significantly fewer caries, with plain milk appearing to be slightly more effective than chocolate milk, which was slightly more effective than chocolate drink. In the filial generation it was found that, regardless of the maternal diet, those rats on the dairy supplement from weaning again had significantly fewer caries than did those maintained on the caries-producing diet. Again, plain milk appeared to be slightly more effective than the other supplements.

In the second experiment, the maternal generation was allowed to have one litter while on the cariogenic diet. It was then divided into three groups of 15 rats each which were either fed the unsupplemented diet or given a 45 per cent caloric supplementation containing 66.3 per cent milk, 23.2 per cent vanilla ice cream and 10.5 per cent cheddar cheese, or the same combination with chocolate instead of plain milk. After three to four weeks on these diets, the rats were bred again and a second litter was produced. Both litters were divided into groups which, from weaning, were fed either the cariogenic diet alone or were fed the dairy products from 21 to 42 days and then transferred to the diet with the 33 per cent dairy supplement from 42 to 106 days.

In addition, some groups were placed on a 45 per cent supplemented diet from weaning to 42 days and a 25 per cent supplemented diet thereafter. Some of this generation were retained on their respective diets for a full 106 days after the 42 day diet

change to allow for an exposure to the cariogenic diet for as long a period as those which had been on the unsupplemented diet from weaning.

It was noted that even in the maternal generation, in which exposure to the supplemented diets occurred after the first litter, there was a slight reduction in the incidence of dental caries. In the filial generation, dental caries was again reduced in all groups which had received the supplementation from weaning. Again, the maternal diet had little or no effect. An increase in caries was noted in those animals fed diets with less supplementation, but not in those which were studied for the longer period of time.

From these experiments, it is clear that a 33 per cent caloric supplementation of a cariogenic diet with various dairy products significantly reduces the incidence of tooth decay in rats. The period immediately after weaning appears to be especially important, since reduction of the 100 per cent supplementation to 45 per cent during this 21-day period resulted in a significant increase in caries in those animals on the plain milk supplementary mixture. The experiments did not, however, determine the nature of this post-developmental protection.

In every case, this effect was found only in the post-eruptive period and the diets of the mothers during the developmental period did not differ in their influence upon the caries activity of their progeny. Presumably this occurred because the diets fed the mothers during pregnancy and lactation were similar with respect to the nutrients required for tooth development and for establishment of caries-susceptibility. The authors point out that the cariogenic diet they used was a generous source of all known inorganic and organic nutrients and that the dairy products fed had no unique ability to promote teeth which were more caries-resistant. If the basal diet had been partially deficient or grossly unbalanced,

supplementation by the levels of dairy products could have resulted in a different situation.

These studies are especially intriguing because of their use of standard human dietary constituents for the prevention of tooth decay. There should be little diffi-

culty in obtaining young human volunteers to subsist on a diet consisting largely of chocolate milk, ice cream and cheese. It would be important to ascertain whether replacement of 33 per cent of the cariogenic diet by other normal human foods will have the same effect.

## DIETARY FAT AND HEPATIC LIPOGENESIS

*Hepatic lipogenesis is found to be depressed by a high fat intake even when the carbohydrate supply is adequate.*

One of the major factors influencing the synthesis of fatty acids by animal cells is the nutritional state of the animal. Definitive information on this subject was provided by G. E. Boxer and DeW. Stetten, Jr. (*J. Biol. Chem.* **153**, 607 (1944)), who found that undernourished rats displayed a very low rate of incorporation of deuterium into body fatty acids. Subsequent work by I. L. Chaikoff *et al.* (*J. Biol. Chem.* **185**, 845 (1950); *Ibid* **196**, 25 (1952)) demonstrated that when rats are fasted or fed a carbohydrate-free diet for a few days, the capacity of liver slices to convert the  $C^{14}$  of  $C^{14}$ -glucose or acetate-1- $C^{14}$  to fatty acids *in vitro* is drastically reduced. Administration of a single dose of glucose, but not of protein or fat, completely restored hepatic lipogenesis to normal in the fasted rat.

The effect of fasting in reducing the lipogenic capacity of liver slices has been observed in experiments with intact rats injected with acetate-1- $C^{14}$  (E. S. West *et al.*, *J. Biol. Chem.* **196**, 389 (1952)). A comparison of the lipogenic capacities of liver, gut, carcass and skin of intact adult rats fasted for various periods of time showed that the lipogenic mechanism in the liver was most susceptible to fasting (West *et al.*, *Ibid.* **208**, 115 (1954)).

The capacity of the liver for lipogenesis is also regulated by hormones (R. O. Brady *et al.*, *J. Biol. Chem.* **193**, 459 (1951)), particularly insulin (Chaikoff *et al.*, *J. Biol. Chem.* **193**, 549, 557 (1951)). The effect of insulin is considered to be secondary to its

action in priming glucose utilization (Chaikoff *et al.*, *J. Biol. Chem.* **197**, 621 (1952)).

R. Hill, J. M. Linazasoro, F. Chevallier and Chaikoff (*J. Biol. Chem.* **233**, 305 (1958)) have also demonstrated an influence of the amount of fat ingested by the rat on hepatic lipogenesis. In one experiment, rats were fed synthetic diets containing 55 per cent of glucose, 22 per cent of casein and 0, 1, 2.5, 5, 10 or 15 per cent of corn oil. Each change in the percentage of fat in the diet was made at the expense of cellulose. The diets were fed for only three days before the animals were sacrificed so that the possibility of appreciably altering liver composition by the prolonged feeding of high fat diets was minimized. The average daily food intakes of the rats were similar, but no attempt was made to keep the caloric value per gram of diet constant.

As the percentage of corn oil in the diet was increased from 0 to 10, the percentage conversion of added acetate-1- $C^{14}$  to fatty acids by liver slices decreased from 20 to about 3.0. No further fall in the extent of conversion occurred in liver slices from rats fed a diet containing 15 per cent of corn oil.

In another experiment, the caloric value per gram of diet was kept from varying greatly by lowering the level of casein in the diet from 37 to 15 per cent as the amount of corn oil was increased from 0 to 15 per cent. The glucose content of these diets was 50 per cent. As before, the average



daily food intakes of rats on these diets were similar. Here again the incorporation of acetate-1-C<sup>14</sup> as well as that of C<sup>14</sup>-glucose into fatty acids was decreased as the fat concentration in the diet was raised.

To show that these results were not influenced by the differences in casein intake, another experiment was carried out in which rats were fed synthetic diets containing no fat and either 15 or 37 per cent of casein. No difference was observed in the capacity of liver slices from these two groups of rats to incorporate acetate-1-C<sup>14</sup> into fatty acids. Also, to show that the effect was not specific for corn oil, a vegetable oil, a hydrogenated vegetable oil and lard were tested. Each was as effective as corn oil in decreasing hepatic lipogenesis from acetate carbon.

In all of these experiments the percentages of added acetate-1-C<sup>14</sup> recovered as C<sup>14</sup>O<sub>2</sub> did not differ significantly from group to group. This, in conjunction with the observation that feeding extra fat did not depress the total utilization of acetate by the liver slices (as estimated by measuring the C<sup>14</sup> remaining in the medium at the end of the incubation period), suggested that the ingestion of fat did not result in a general depression of the metabolic activity of the liver, but rather in a specific inhibition of lipogenesis.

The effects of high levels of fat in the diet on the lipogenic capacity of the liver were also studied in experiments with intact rats. The animals were fed isocaloric diets containing 0, 5, 10, or 15 per cent of corn oil, for three days. They were then injected with acetate-1-C<sup>14</sup> and killed one

hour later. The livers were removed and analyzed for C<sup>14</sup>-fatty acids. Again the percentage of C<sup>14</sup>-fatty acids recovered from the liver decreased as the amount of fat in the diet increased.

These effects of ingested fat upon hepatic lipogenesis occurred without changes in the glycogen and fat content of the liver or in the glucose and lipid content of plasma. Also, in no case was there a decrease in the glycogen content of the liver of rats fed the high fat diets for three days. "Thus, the depressing effect of dietary fat upon hepatic lipogenesis was observed under conditions that allowed for priming of hepatic lipogenesis by carbohydrate."

In view of the influence of insulin on hepatic lipogenesis, the possibility that the depression in lipogenesis produced by a high fat diet might be a result of a relative decrease in available hormone was examined. In normal rats fed on diets containing 50 per cent of glucose, with or without added fat, the administration of two units of insulin twice daily for three days failed to prevent the depression in hepatic lipogenesis induced by a high fat intake.

The results of these experiments indicate that the lipogenic activity of the liver is controlled in some way by the amount of fat ingested and that hepatic lipogenesis is depressed by a high fat intake, even when the diet contains a high percentage of carbohydrate. The mechanism of this adaptive response remains to be explained, but it does not appear to be mediated by the depressing effect of a high-fat intake on insulin production.

### AN ENZYMATIC FUNCTION OF CARNITINE

*With palmitate (but not shorter chain fatty acids) the addition of carnitine to rat liver homogenates increases the formation of ketone bodies but has no influence on carbon dioxide production.*

The recognition that carnitine was required for the growth of the meal worm (*Nutrition Reviews* 13, 24 (1955)) stimu-

lated work to determine the metabolic role of this compound. Although only very small amounts of carnitine are required for

the growth of the meal worm, the tissues of higher animals contain fairly large amounts of it. Skeletal muscle contains the highest concentration of carnitine (560 to 1120 micrograms per gram).

G. Fraenkel (*Nutrition Reviews*, *loc. cit.*) showed that carnitine was in the developing chick, appearing fairly early and increasing in content as the embryo matured. No carnitine could be detected in the unfertilized egg.

During the past few years I. B. Fritz has investigated the role of carnitine in the intact rat and in enzymatic preparations of livers removed from these animals. He was led to this work by E. Lundsgaard, who found evidence for a factor in blood and muscle extract which increased the oxygen consumption of perfused cat livers (*Biochim. Biophys. Acta* **4**, 322 (1950)).

Fritz (*Acta Physiol. Scandinav.* **34**, 367 (1955)) used liver slices and homogenate preparations instead of the perfused whole liver. He found that when either labeled glucose, acetate or palmitate was the substrate, the production of labeled ketone bodies was increased by the factor in blood and muscle extract. Carnitine could partially replace the blood or muscle extract.

Later Fritz was intrigued by the observation of C. Artom (*J. Biol. Chem.* **213**, 681 (1955)) that the livers of choline-deficient rats oxidized labeled fatty acids at a slower rate than did similar preparations from normal animals. The similarity in the structure of choline and carnitine suggested to Fritz that choline might be a precursor of carnitine or a carnitine derivative.

To test the above hypothesis, he put weanling rats on choline-deficient diets containing either 5 or 20 per cent casein (*Am. J. Physiol.* **190**, 449 (1957)). The livers from the choline-deficient animals were homogenized and added to a buffer containing inorganic salts, adenosine triphosphate and palmitic-1-C<sup>14</sup> acid. Carnitine at a concentration of  $6.7 \times 10^{-5}$  molar was added to half of the Warburg flasks. The presence of this amount of carnitine (30

micrograms in 4.5 ml. of medium) increased the formation of ketone bodies over that seen in the control flasks without the added carnitine (513 counts versus 214 counts per minute per mg. of dry fat-free liver). The carnitine had no effect on the production of carbon dioxide in this system. Livers from the control rats fed a commercial stock diet as well as those from the choline-supplemented animals showed a similar response.

The above observations led Fritz to conclude that choline is not converted to carnitine or a carnitine derivative.

Another observation made by Fritz (*loc. cit.*) was that the liver homogenates from the choline-supplemented rats showed a greater carbon dioxide production than those from the deficient rats. In these experiments, labeled palmitic acid was also the substrate. Since the homogenates from the livers of both the supplemented and deficient rats still responded to the addition of carnitine with an increase in ketone production, Fritz suggested that choline and carnitine "influence different parameters of fat metabolism."

In an accompanying paper, I. B. Fritz and P. DuPont (*Am. J. Physiol.* **190**, 453 (1957)) reported that carnitine could not replace choline as a lipotropic factor. In these studies they used weanling rats maintained on a choline-deficient diet containing 20 per cent protein. On this diet, the rats developed livers containing 16 per cent fat. In another group of rats the addition of 2.6 g. of choline chloride per kg. of diet reduced the fat level of the livers to 5.3 per cent. The addition of 3.4 g. of DL-carnitine chloride to one kg. of the choline-deficient diet resulted in fat levels of 13.8 per cent in the livers of a third group of rats.

In an attempt to elucidate the mechanism whereby carnitine increased the ketone body production of liver homogenates, Fritz carried out a variety of enzymatic studies (*Am. J. Physiol.* **197**, 297 (1959)). He prepared liver slices from male rats

weighing about 200 g. and incubated them with inorganic salts. To half of the Warburg vessels, carnitine was added at a level of  $3 \times 10^{-4}$  molar. When labeled octanoate was present in the flasks, there was no increase in the formation of carbon dioxide or of ketone bodies. However, when either palmitate or stearate, both labeled in the carboxyl group, were incubated with the slices, there was an increased ketone production (110 to 140 per cent over that of the controls) but no change in the carbon dioxide production.

He prepared a liver particulate fraction which represented 25 per cent of the original liver weight. When this was incubated with labeled butyrate, octanoate or laurate, there was no effect of carnitine addition on the production of carbon dioxide or the formation of ketones. Again, in the presence of palmitate or stearate, carnitine increased the formation of ketones by a factor of 2 to 4.

The effect of carnitine in increasing the formation of ketone bodies by liver preparations incubated with palmitic acid was seen at several different levels that were roughly comparable to those which might exist in serum, if all the unesterified fatty acids were present as palmitate ( $10^{-4}$  to  $10^{-3}$  molar). When the level of palmitate was held constant, there was a direct relation between the formation of ketone bodies and the amount of carnitine added to the Warburg

flask. The relationship was essentially linear over the range from 1.0 to 100.0 micrograms.

The observed effect of carnitine was not related to the formation of palmityl-coenzyme A. This was shown by the observation that carnitine did not enhance the formation of ketone bodies when the palmityl-coenzyme A was incubated with rat liver particulates. The preceding finding suggested that carnitine probably influenced some reaction prior to the formation of palmityl-coenzyme A. To test this possibility, enzymatic studies were carried out with hydroxylamine which traps the activated fatty acids (*i.e.*, as the coenzyme A complex). The addition of carnitine was without effect on the amount of palmityl hydroxamate formed. Fritz was led to conclude that "carnitine influences long chain fatty acid oxidation at a level prior to the formation of the coenzyme A derivative in a system which retains some semblance of intracellular structure."

Whether the effect of carnitine in increasing the formation of ketone bodies from long chain fatty acids is an artifact or represents an important physiological function will remain for future research. The observations of Fritz provide an important clue and suggest strongly that carnitine may be important in some phase of the metabolism of long chain fatty acids.

## MOLYBDENUM TOXICITY

*It is possible that molybdenum exerts its toxic action by virtue of an effect on certain enzyme systems rather than by a direct effect on mineral and chondroitin sulfate deposition in the bones.*

Cattle, rabbits and chicks fed high dietary levels of molybdenum all show symptoms that include deformities of the joints of extremities, reduction in the rates of growth, anemia and diarrhea (W. S. Ferguson *et al.*,

*J. Agr. Sci.* **33**, 40 (1943); L. R. Arrington and G. K. Davis, *J. Nutrition* **51**, 295 (1953); and B. L. Reid *et al.*, *Fed. Proc.* **17**, 294 (1958)). The bone deformity is strongly suggestive of a disturbance in calcium and

phosphorus metabolism, but R. F. Miller and N. O. Price (*Fed. Proc.* **15**, 564 (1956)) have reported that the addition of inorganic sulfate to high molybdenum diets alleviates the growth depressing effects of the molybdenum. Thus, the deformities were suspected to be due to an imposed failure of the organic phase of bone metabolism.

In order to resolve this question, J. P. Feaster and G. K. Davis (*J. Nutrition* **67**, 319, 325 (1959)) have carried out nutritional experiments on growing rabbits employing radioactive sulfate, calcium and phosphorus.

In the studies treating calcium and phosphorus metabolism, one group of rabbits was fed a control diet of rolled oats (88 per cent) alfalfa meal (10 per cent) and sucrose (2 per cent), with calcium acetate added to furnish the proper calcium to phosphorus ratios. The second group received the same basal diet with molybdenum added at a level of 0.2 per cent as sodium molybdate. After the animals had been fed these diets for five weeks, each received a single oral dose containing 3 microcuries of  $\text{Ca}^{45}$  and  $\text{P}^{32}$ .

Three rabbits from each lot were killed after intervals of six, 24 and 48 hours and seven and 14 days. Blood, liver, kidney, skeletal muscle, cardiac muscle and bone were taken for determinations of both radioactive and stable calcium and phosphorus. Urine and feces collected at 24, 48, 96 and 168 hours after administration of the radioactive materials were also analyzed for calcium and phosphorus.

Although food consumption by the rabbits receiving molybdenum was slightly lower than that of the control rabbits, the difference in weights of the animals at sacrifice could be only partially accounted for on this basis. All symptoms of molybdenum toxicity as mentioned above were present.

There was no appreciable effect of the level of molybdenum in the diet on uptake of radioactive calcium and phosphorus in the soft tissues. Amounts of  $\text{Ca}^{45}$  and  $\text{P}^{32}$

excreted by the control rabbits exceeded only slightly the amounts excreted by the high-molybdenum rabbits, except in the fecal excretion of  $\text{P}^{32}$ . Rabbits of the control group also excreted more total calcium and phosphorus in both urine and feces than those of the high-molybdenum group, because of higher feed consumption by the control animals.

In view of the skeletal deformities observed in the rabbits on high-molybdenum intake, it was expected that bone would show the greatest alterations in calcium and phosphorus content. Decreased contents of stable calcium and phosphorus and decreased uptake of  $\text{Ca}^{45}$  and  $\text{P}^{32}$  were anticipated. Nevertheless, analyses of the humeri did not bear out this prediction. The uptake of  $\text{Ca}^{45}$  was slightly greater in the humeri of the high-molybdenum rabbits than in the controls and apparently continued over a longer period of time. While the uptake of  $\text{P}^{32}$  was less rapid in the humeri of the high-molybdenum rabbits during the first six hours after administration,  $\text{P}^{32}$  levels in these animals exceeded control levels between 24 and 48 hours after administration of the isotopes and at 14 days were nearly twice as high as control  $\text{P}^{32}$  values. Absolute amounts, as well as concentrations, of  $\text{Ca}^{45}$  and  $\text{P}^{32}$  were higher in the humeri of the high-molybdenum rabbits than in the controls. Thus, an increased uptake of these elements was seen to have occurred following ingestion of relatively large amounts of molybdenum.

Weights of humeri from the high-molybdenum animals sacrificed two weeks after isotope administration averaged 2.92 g. as compared with 5.48 g. in control animals. Yet, the total amounts of calcium and phosphorus present in the smaller bones from the molybdenum-treated rabbits were nearly as high as those in control bones. It is, therefore, presumed that the decrease in weight of the humeri was the result of diminution of organic matter. Part of this

was probably due to decreases in marrow since anemia is a common result of molybdenum toxicity. The authors report that H. F. Roberts (*Ph.D. Dissertation, University of Florida, 1956*), had observed areas of necrosis in the marrow and a decrease in the number of nucleated red blood cells in the blood sinuses.

Although Roberts (*loc. cit.*) had noted that there was no evidence of defective deposition of inorganic materials in the cartilage matrix, Feaster and Davis carried out a series of studies to determine what effect a high level of molybdenum in the diet has on the tissue distribution and excretion of radioactive sulfate. The rabbits were fed control and experimental diets similar to those that had provided the data on calcium and phosphorus metabolism. After the rabbits had been on these diets for five weeks, each received a single oral dose of 10 microcuries of  $S^{35}$  in the form of sodium sulfate. Tissues taken for determinations of both radioactive and stable sulfur included liver, muscle, bone and hair. Urine and feces were also analyzed.

Levels of  $S^{35}$  found in the livers and bones of these rabbits were lower than in rabbits maintained on the control diet. Urinary excretion of  $S^{35}$  was higher, indicating decreased retention of sulfur in the high-molybdenum animals. Yet, in the humeri, alone, total sulfur content was significantly higher than in control rabbits, indicating a higher proportion of cartilage. (Most of the sulfur present in the skeleton occurs in the cartilage as chondroitin sulfate.)

Because sulfur usually serves as an indicator of protein metabolism, it is possible

that the excretion of a higher amount of urinary sulfur indicated a lowered protein metabolism in molybdenum fed animals. Analyses of the urines for nitrogen revealed an average excretion of 27 mg. per control rabbit, compared with 63 mg. for the high-molybdenum rabbits. The authors are of the opinion that if the control rabbits are considered to be in nitrogen balance, or even slightly on the positive side since they were in the growing stage, then the high-molybdenum rabbits were probably in negative nitrogen balance. In wasting accompanying starvation, the urinary nitrogen to sulfur ratio remains virtually unchanged. However, in the present study the nitrogen to sulfur ratio of the high molybdenum rabbits was twice as high as that of the controls, indicating toxic effects of the high intake of molybdenum over and above those due to decreased food intake.

According to these investigators, it is possible that molybdenum exerts its toxic action by virtue of an effect on certain enzyme systems. M. Bossard (*Bull. Soc. Chim. Biol.* 29, 218 (1947)) noted that sodium molybdate inhibited all phosphatases found in extracts of plant tissues. On the other hand, D. A. Richert and W. W. Westerfeld (*J. Biol. Chem.* 203, 915 (1953)) have isolated from soy flour a molybdenum salt essential to the deposition and maintenance of normal levels of rat intestinal xanthine oxidase. Thus, it appears that molybdenum, up to certain concentrations, may be necessary to the action of certain enzymes, but beyond these levels may act as inhibitors of these or other enzymes.

## DIETARY FATTY ACID INTERRELATIONSHIPS

*Essential fatty acids are required for proper utilization of fat calories. High ratios of saturated fats may promote essential fatty acid deficiency symptoms.*

Although nutritionists have been concerned for many years with the optimum amount and composition of fat for the human diet, the most that can be said with

any certainty at the present time is that the optimum lies between the limits of the very small amount necessary to supply sufficient essential fatty acids and the excessive amounts which appear to lead to hyperlipemia and, possibly, to atherosclerosis. Aside from the question of adequate essential fatty acid intake, but inevitably tied to the problem of total dietary fat, is that of the optimum proportion of saturated and unsaturated fat. Some consideration has been given to this problem (*Nutrition Reviews* 14, 305 (1956)) but the result has been only a start in the right direction.

To further complicate the entire picture, it may be recalled that Greenberg and co-workers found that, although male rats which had been on a fat-free diet from weaning maintained a fairly normal growth rate when given a supplement of 100 mg. of methyl linoleate per day, they grew even faster when this supplement was given in cottonseed oil (S. M. Greenberg *et al.*, *J. Nutrition* 41, 473 (1950); 45, 521 (1951)). Evidently, either the cottonseed oil had additional growth promoting factors or it acted in a complementary manner with the linoleate. It should be stressed, however, that growth rate may not be a sufficient criterion for dietary excellence (*Nutrition Reviews* 16, 350 (1958)).

The relationship between the essential fatty acid content of the diet and total saturated fat has recently been studied by J. G. Peifer and R. T. Holman (*J. Nutrition* 68, 155 (1959)). Two separate experiments were performed. In the first, hydrogenated coconut oil and ethyl esters from corn oil were fed to male weanling rats in various proportions as part of an otherwise adequate diet. With 1 per cent corn oil and 9 per cent hydrogenated coconut oil, growth was significantly better than with 1 per cent of either hydrogenated coconut oil or corn oil or with 10 per cent hydrogenated coconut oil. Dermal symptoms, which were also studied, were absent in both groups fed corn oil, but very significant in its absence.

In the second experiment, various proportions of hydrogenated coconut oil and ethyl linoleate up to a total of 25 per cent of the diet weight (or 47.6 per cent of the calories) were fed. In this case, it was observed that in the absence of ethyl linoleate, growth rates were decreased when the hydrogenated coconut oil was increased from 1 to 25 per cent. Dermal symptoms, however, decreased on increasing the coconut oil (perhaps as a result of the decreased growth rate). When 0.5 per cent ethyl linoleate was supplied, however, 24.5 (but not 9.5 per cent) hydrogenated coconut oil appeared to be somewhat growth inhibiting. Caloric efficiencies were considerably lower for rats on the essential fatty acid deficient diets and varied inversely with the coconut oil content.

Analysis of the polyunsaturated acids of the hearts of animals in this experiment revealed that as the hydrogenated coconut oil content of the diet was increased, the heart polyunsaturated acids of all types increased. Since, in half the rats no linoleate was being ingested, the source of this acid and the higher polyunsaturated acids elaborated from it must have been in the other tissues. This point, however, was not investigated in these studies. In particular, trienoic acids, which are known to accumulate in the fat deficiency state (*Nutrition Reviews* 15, 86 (1957)), were very high in the animals on the ethyl linoleate-deficient diets. With the linoleate-containing diets, increasing the hydrogenated coconut oil content to 24.5 per cent resulted in a manifold increase in the trienoic acid (to about 30 per cent of the values for linoleate-deficient diets). This suggested that a change in the direction of essential fatty acid deficiency had been produced. Tetraenoic, pentaenoic and hexaenoic acids tended to increase on the diets high in hydrogenated coconut oil, but, unlike the trienoic acid, the values were generally greater when the diet contained ethyl linoleate.

In general, although the total endogenous polyunsaturated acids (all the above minus linoleate) tended to increase in the hearts with an increase in the dietary saturated fat, the relative amounts of these acids remained fairly constant. The exception was the increase in the relative amounts of trienoic acid in the rats fed ethyl linoleate with increasing hydrogenated coconut oil. This points toward a possible competitive dehydrogenation of the saturated fatty acids which were fed in increasing proportions, producing eicosatrienoic acids from the dehydrogenation of palmitic and stearic acids.

The authors conclude that with an adequate intake of essential fatty acids, increased dietary saturated fat stimulates growth rate. In essential fatty acid deficient animals, however, the saturated fat is not used for growth. It appears, then, that the essential fatty acids are in some way necessary for proper utilization of the saturated fatty acids. Three possible reasons were

suggested as to why increased dietary saturated fat stimulated an increased polyunsaturated fatty acid content in the heart. These were (1) a sparing action of saturated fat on the catabolism of heart polyunsaturated acids, (2) a mobilization of polyunsaturated acids to the heart, and (3) a stimulating effect of high levels of dietary saturated fat on polyunsaturated fatty acid synthesis. In this latter consideration it is possible that the tetraenoic acid synthesized by the ethyl linoleate-deficient animals may not be arachidonic.

These considerations lead to the conclusion that the fat content of the diet must be studied with respect to the ratios of the different types of fatty acids present and their interactions with each other. It will be important now to study the relationships of the mono-unsaturated acids, oleic and palmitoleic, the most prevalent class of dietary fatty acids, which may not behave like either the saturated or polyunsaturated classes.

### METHIONINE METABOLISM

*The substitution or supplementation of methionine with choline, folacin, homocystine and vitamin B<sub>12</sub> in the diet of the mouse has relatively little effect.*

Numerous studies in experimental animals, including the rat, chick and other species, have established the existence of relationships among methionine, choline, folic acid and vitamin B<sub>12</sub> (A. E. Schaefer and J. L. Knowles, *Proc. Soc. Exp. Biol. Med.* **77**, 655 (1951); M. A. Bennett, *J. Biol. Chem.* **187**, 751 (1950); J. A. Stekol *et al.*, *Arch. Biochem. Biophys.* **36**, 5 (1952); T. H. Jukes and E. L. R. Stokstad, *J. Nutrition* **48**, 209 (1952)). These relationships apparently involve the synthesis, transfer and utilization of methyl groups.

Relatively few studies of these relationships have been carried out in mice. E. Nielsen and A. Black (*J. Nutrition* **28**, 203 (1944)) have obtained some evidence that

the mouse requires folacin for growth and reproduction. Anti-folacin compounds produce growth depressions in mice that may be reversed with folacin or leucovorin (H. P. Broquist *et al.*, *J. Nutrition* **47**, 93 (1952)).

In conjunction with the use of thyroid-active substances or high protein diets, vitamin B<sub>12</sub> has been shown to stimulate the growth of mice (D. K. Bosshardt *et al.*, *J. Nutrition* **40**, 595 (1950)). Y. C. P. Lee *et al.* (*Am. J. Physiol.* **173**, 456 (1953)) reported that fetal resorption in the mouse could be prevented with either alpha-tocopherol or vitamin B<sub>12</sub> supplementation.

Since several investigations had indicated that the mouse was more resistant to a choline deficiency than the rat or chick



(H. M. Barret *et al.*, *J. Physiol.* **93**, 367 (1938)), H. E. Sauberlich (*J. Nutrition* **68**, 141 (1959)) has made a concentrated study of the possible relationships of methionine, choline, folacin and vitamin B<sub>12</sub> in the mouse.

Commercial weanling male albino mice of the Rockland Swiss-Webster strain were given food and water ad libitum and weighed twice a week. A methionine- and methyl-free basal diet was used in nearly all experiments consisting of 12 per cent oxidized casein or methanol-extracted casein, 4 per cent corn oil, 1 per cent cod liver oil, 4 per cent salts, 72.5 per cent sucrose, 0.3 per cent L-cystine, and 0.2 per cent DL-tryptophan. Adequate amounts of inositol, calcium pantothenate, alpha-tocopherol, alpha-tocopherol acetate, niacin, riboflavin, pyridoxine, thiamine, 2-methyl-1, 4-naphthoquinone and biotin were added routinely, and supplements of varying amounts of choline, homocystine, betaine, folacin, vitamin B<sub>12</sub>, methionine and other amino acids constituted the experimental procedure. In some of the animals, fat content and the transmethyrase and choline oxidase activities of the liver were determined.

Mice fed a methionine-free, choline-free basal diet, supplemented with homocystine, died or failed to grow when either folacin or vitamin B<sub>12</sub> was added alone. The addition of both vitamins permitted survival but resulted in little growth. Further supplementation with glycine, serine and threonine resulted in growth about 50 per cent of normal.

When choline alone was added to the homocystine-supplemented basal diet, only slight growth resulted. On the other hand, about 50 per cent of normal growth was achieved with betaine. When folacin was added in either of the situations cited above, nearly normal growth resulted and the further addition of vitamin B<sub>12</sub> had only a slight effect. Methionine alone permitted nearly normal growth, which was improved

slightly by the addition of choline, folacin and vitamin B<sub>12</sub>.

The observation that homocystine, in the absence of a methyl source, required both folacin and vitamin B<sub>12</sub> to permit survival and limited growth would indicate that these vitamins are involved in the synthesis, transfer or utilization of methyl groups. Studies with other species indicate that vitamin B<sub>12</sub> aids in the synthesis of methyl groups, while folacin is involved in certain transfers of methyl groups.

Apparently the requirement for folacin in the methylation of homocysteine is reduced when betaine is the source of methyl groups rather than choline. The author explains this on the basis that the requirement for folacin in order to maintain choline oxidase activity in the conversion of choline to betaine, as demonstrated in the rat and the chick, would be avoided. Folacin may still be required for the betaine-homocystine transmethyrase reaction.

Sauberlich found that liver choline oxidase activity was markedly reduced in mice fed the basal diet supplemented with homocystine alone. The reduction in activity was largely prevented by dietary supplementation with folacin and, in part, by supplementation with vitamin B<sub>12</sub>. Choline was also effective but betaine was not.

Transmethyrase activity of the liver was reduced when choline, folacin and vitamin B<sub>12</sub> were omitted from the basal homocystine diet. When folacin and vitamin B<sub>12</sub> were added to the diet the activity was improved. Choline and folacin added together gave normal transmethyrase activity, while only partial improvement was noted following addition of choline and vitamin B<sub>12</sub>. Thus the importance for the mouse of vitamin B<sub>12</sub> in relation to this activity is not certain. The effect of vitamin B<sub>12</sub> may be a reflection of needs for methyl groups that could be furnished by *de novo* synthesis in the presence of vitamin B<sub>12</sub> but could not be fur-

nished by choline except in the presence of folacin.

The author noted that extremely fatty livers were produced in mice fed the basal homocystine diet, either in the presence or

absence of folacin and vitamin B<sub>12</sub>. The presence of choline or betaine largely prevented this condition, and supplementation with choline, folacin and vitamin B<sub>12</sub> resulted in normal liver fat levels.

### RIBOFLAVIN REQUIREMENTS OF THE CAT

*In the cat, as in man, the riboflavin requirement is increased by a high-fat diet and decreased by a high-carbohydrate diet.*

Little is known of the nutritional requirements of the domestic cat. In an initial attempt to close this hiatus in our knowledge of the nutrition of laboratory animals, S. N. Gershoff, S. B. Andrus and D. M. Hegsted (*J. Nutrition* **68**, 75 (1959)) have studied the riboflavin requirements of cats fed diets varying in carbohydrate and fat content.

Three- to six-months-old kittens of mixed breed and sex were used. The two diets employed in the studies differed considerably in their fat and carbohydrate content. In the high-fat diet 46 per cent of the calories came from fat and 29 per cent from carbohydrates, and in the high-carbohydrate diet 11 per cent of the calories were from fat and 64 per cent from carbohydrates. In both diets, 25 per cent of the calories were provided by casein.

Two separate experiments were carried out. In the first, nine kittens were fed the low-carbohydrate, high-fat diet without riboflavin for two months, by which time all were suffering from riboflavin deficiency. Four cats were sacrificed for histological study. The others were given 5 mg. of riboflavin subcutaneously and then fed diets with graded amounts of riboflavin. Two cats received 0.5 mg., two cats 1.0 mg., and one cat 1.5 mg. of riboflavin per kg. of diet. All of these cats died, presumably of riboflavin deficiency, within four months of starting these diets and were autopsied within two hours following death.

In a second experiment, cats were fed both experimental diets with varying amounts of

riboflavin for periods up to 34 months. Acute deficiency in cats fed diets without added riboflavin resulted in anorexia accompanied by weight loss terminating in death. In the initial experiment, seven of nine cats showed some loss of hair about the eyes and ears after two months of the un-supplemented diet.

In acute riboflavin deficiency, those animals fed the high-fat diet became moribund sooner than those fed the high-carbohydrate diet. Chronic riboflavin deficiency differed from acute deficiency not only in a more protracted course but also in the occurrence of cataracts. All five animals that developed cataracts were receiving the high-fat diet. These cataracts occurred some months after the vitamin-deficient diet was started. In none of the cats in either experiment were there lesions of the cornea, changes about the mouth, anemia, paralysis, or the riboflavin collapse syndrome.

Fat was generally present in the parenchymal cells of the livers of deficient cats. All the male cats, whose testes were studied histologically, showed distinct testicular hypoplasia. There was generally complete aspermia and decreased numbers of spermatids and spermatocytes. No alterations of the myelin structures of the brain or spinal cord were observed. Skin changes were less dramatic than for other species.

Two cats remained in excellent health for more than 34 months on the high-carbohydrate diet supplemented with 3 mg. of riboflavin per kg. Cats were routinely

maintained in the laboratory on the low-carbohydrate diet plus 4 mg. of riboflavin per kg. for periods up to two years.

Urinary and fecal riboflavin analyses revealed that the better maintenance on the high-carbohydrate diet was caused by greater synthesis of riboflavin in these animals. Since synthesis was high on both diets, the authors concluded that the effect was due to both increased intestinal synthesis and utilization.

These results are in contrast to observations of R. L. Potter *et al.* (*J. Nutrition* **24**, 449 (1942)) on dogs, and of G. J. Mannering *et al.* (*Proc. Soc. Exp. Biol. Med.*, **46**, 100 (1941)) on rats. None of the latter workers was able to detect a sparing action of sucrose on the dietary requirements for riboflavin. Riboflavin deficiency has, however, been associated with cataracts in the rat (P. L. Day and W. C. Langston, *J. Nutrition* **7**, 97 (1934)), the mouse (S. W. Lippincott and H. P. Morris, *J. Nat. Cancer Inst.* **2**, 601 (1941)) and the pig (A. J. Patek, Jr., *et al.*, *Am. J. Physiol.* **133**, 47 (1941)).

The actual incidence of cataract production in the rat is highly variable, ranging from 0 to 100 per cent. Fatty livers have been described in riboflavin-deficient dogs, although one of the groups studying this phase of riboflavin deficiency (H. R. Street *et al.*, *J. Nutrition* **22**, 7 (1941)) concluded after further work that such changes could

be explained on the basis of inanition. Partial paralysis because of myelin degeneration of the control nervous system and peripheral nerves, so prominent in other species (H. M. Zimmerman and E. Burack, *J. Exp. Med.* **59**, 21 (1934); J. H. Shaw and P. H. Phillips, *J. Nutrition*, **22**, 345 (1941); L. D. Greenberg and J. F. Rinehart, *Fed. Proc.* **15**, 265 (1956)) were not observed in riboflavin-deficient cats.

B. Bro-Rasmussen (*Nutrition Abst. Rev.* **28**, 1, 369 (1958)) has summarized and evaluated much of the data available on the riboflavin requirements of different species and has concluded that the minimal requirement of different species is the same if expressed upon a calorie basis. The minimum need of adult man is, by this yardstick, concluded to be approximately 0.25 mg. per 1000 calories, and 0.5 to 0.6 mg. per 1000 calories is assumed to be an adequate intake. Bro-Rasmussen also concludes that the intestinal flora may be of importance for man's riboflavin supply.

Gershoff, Andrus and Hegsted (*loc. cit.*) also concluded that the studies of E. M. Widdowson and R. A. McCance (*Medical Research Council Special Report Series*, No. 287 London, 1954) in German orphanages provide substantial evidence that the intestinal synthesis and the urinary excretion of riboflavin depend on the ratio of fat to carbohydrate calories in the diet.

## UTILIZATION OF HYDROXYANTHRANILATE

*The evidence presented supports the view that 3-hydroxyanthranilic acid is an intermediate in the conversion of tryptophan to niacin in guinea pigs, chicks and hamsters.*

It is well known that animal species differ in their dietary requirement for niacin. These differences probably result in large measure from the fact that tryptophan substitutes for this vitamin in varying degrees in different species (W. A. Krehl *et al.*, *Science* **101**, 283, 489 (1945)). The general pattern of biosynthesis of niacin from tryp-

tophan by *Neurospora crassa* has been well established, and there is abundant evidence that this pathway occurs in mammals. H. K. Mitchell and J. F. Nyc (*Proc. Nat. Acad. Sci.* **34**, 1 (1948)) have identified 3-hydroxyanthranilic acid as an intermediate in this conversion in studies with *Neurospora*. Its niacin-replacing activity in the rat is



approximately equal to that of tryptophan or about 2 per cent of that of niacin (Mitchell *et al.*, *J. Biol. Chem.* **175**, 433 (1948)).

D. H. Decker and L. M. Henderson (*J. Nutrition* **68**, 17 (1959)) point out, however, that *in vitro* studies, chiefly with rat tissues, have not confirmed observations made in the intact rat that oxidation of 3-hydroxyanthranilate by slices and cell-free preparations has failed to produce niacin (A. H. Bokman and B. S. Schweigert, *Arch. Biochem. Biophys.* **33**, 270 (1951); Henderson and H. M. Hirsch, *J. Biol. Chem.* **181**, 667 (1949); A. H. Mehler, *Ibid.* **218**, 241 (1956)).

However, according to R. J. Suhbadolnik *et al.* (*J. Biol. Chem.* **228**, 973 (1957)) the liver filtrates from ten different mammalian species varied considerably in the rate at which they oxidized 3-hydroxyanthranilic acid, the liver of the rat containing less of the 3-hydroxyanthranilic acid oxidase than most of the species studied.

Decker and Henderson (*loc. cit.*) therefore decided that the results of *in vitro* studies must be checked by carrying out determinations *in vivo* on each species that had been studied *in vitro*. In an initial growth study, three species, the chick, the guinea pig and the hamster, were characterized with respect to their efficiency in substituting 3-hydroxyanthranilate for niacin.

Since so many species appear to utilize tryptophan as a source of niacin, the diets were prepared so as to make this the limiting amino acid. All amino acids, except tryptophan, were provided at levels in excess of the minimum requirement by the addition of proteins low in tryptophan such as corn proteins and gelatin.

Data presented by G. M. Briggs *et al.* (*Proc. Soc. Exp. Biol. Med.* **51**, 59 (1942)) indicated that the efficiency of conversion of tryptophan in the chick is about 4 per cent (the chick could grow about as well on a niacin-free diet supplemented with 100 mg. per cent tryptophan as on the same diet supplemented with 2.5 mg. per

cent niacin). The level of 3-hydroxyanthranilic acid selected by Decker and Henderson for their experiments was 80 mg. per cent. The results indicated that while 3-hydroxyanthranilic acid is used to replace niacin, it is not as effective at 80 mg. per cent as niacin is at 2.5 mg. per cent. Thus, according to these experiments, 3-hydroxyanthranilate has less than 4 per cent of the effectiveness of niacin in supporting growth in chicks.

Their results with the guinea pig agreed well with those appearing in the literature (M. D. Cannon *et al.*, *Proc. Soc. Exp. Biol. Med.* **63**, 414 (1946)). When 100, 200 and 300 mg. per cent of 3-hydroxyanthranilic acid were fed, Decker and Henderson found that 300 mg. per cent gave better growth than either of the two lower levels and was also more effective than 3 mg. per cent of niacin. They point out that 3-hydroxyanthranilic acid has a niacin-replacing activity in the guinea pig approximately equal to that of tryptophan.

In preliminary experiments, three levels of 3-hydroxyanthranilic acid were tested in hamsters. Seventy-five mg. per cent gave better growth than two lower levels and supported growth comparable to that obtained with 1.5 mg. of niacin. The authors report that the hamsters were markedly affected by niacin deficiency. During the first 12 days of the growth studies, the weights of the animals in all three groups were about the same, but by the end of the second week losses of weight were observed in all the negative control animals. Two died on the eighteenth day and two others developed diarrhea and unkempt fur.

The authors conclude that 3-hydroxyanthranilate has approximately the same niacin-replacing activity as tryptophan in the chick, guinea pig and hamster. They are of the opinion that the evidence presented supports the view that in these species 3-hydroxyanthranilic acid is an intermediate in the conversion of tryptophan to niacin.

## NOTES

## Letter to the Editor

Dear Sir:

The thiamine sparing action of some antibiotics (penicillin) is due to their action on the intestinal flora which produce thiamine (*Nutrition Reviews* 17, 151 (1959)). It is not known whether the thiamine synthesized by the intestinal flora is absorbed during "first passage" or during "second passage" after coprophagy. We should like to mention some experiments concerning this question (*Int. Rev. Vitamin Res.* 30, 376 (1959)).

Similar to penicillin, sorbitol was also found to be thiamine sparing, as shown by British, American and German workers. The vitamin sparing action was abolished by addition of sulfaguanidine to the semi-synthetic diet containing sorbitol. Furthermore, there was no sparing action during complete prevention of coprophagy, whereas the feeding of feces collected during this time caused a significant increase of body weight.

These results may indicate that extra thiamine produced by stimulation of the intestinal microbiological synthesis by sorbitol is not absorbed during "first passage", but becomes available after coprophagy. This hypothesis corresponds with our experiments on humans, since no thiamine sparing action of sorbitol was demonstrated.

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DIETER HÖTZEL  
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## Vitamin Interrelationships

The July-August number of the *American Journal of Clinical Nutrition* (7, 375-443 (1959)) is very largely devoted to the papers presented at a Symposium on Vitamin Interrelationships held at the Medical College of Virginia on October 24, 1958.

Of the nine papers presented, four were concerned with folic acid and other pteridines. These covered the following subjects: the role of biopterin (2-amino, 4-hydroxy, 6-dihydroxypropyl-pteridine) in the nutrition and metabolism of the protozoan parasite, *Crithidia fasciculata*, and its relationship to other growth factors, discussed by H. A. Nathan and H. B. Funk; hematopoietic effects of metabolites that depend for their synthesis upon coenzymes containing folic acid, discussed by R. W. Rundles; the production of an uncomplicated deficiency of folic acid in mice by dietary means, presented by G. M. Briggs; and formiminoglutamic acid excretion following a loading dose of histidine, a diagnostic test for folic acid deficiency, presented by A. L. Luhby, J. M. Cooperman and D. N. Teller.

The major emphasis in the remaining papers was on pyridoxine, pantothenic acid and vitamin B<sub>12</sub> relationships, with a paper on the effects of pyridoxine and pantothenic acid deficiencies on conditioned reflexes by W. H. Gantt, B. F. Chow and M. Simonson, and one on the effect of pyridoxine deficiency on vitamin B<sub>12</sub> absorption in rats by S. D. J. Yeh and Chow. Vitamin interrelationships in heme synthesis were discussed by D. A. Richert and M. P. Schulman. The role of intrinsic factor in vitamin B<sub>12</sub> absorption, transport and storage was discussed by V. Herbert.

The remaining paper was a short, speculative but stimulating appraisal by five scientists from the Haskins Laboratories and the Department of Chemistry, Mount Sinai Hospital, New York, of the outlook for studying vitamin interrelationships by microbiological methods.

These papers provide a survey of some of the more recent work on vitamin interrelationships. It is a service to nutritionists to have them published together.

### Decreased Density of Bone: An Etiologic Approach to Diagnosis

Recently W. H. Meroney, M. E. Rubini, P. J. Rosch, F. K. Austen, E. G. Herndon, Jr. and W. B. Blythe (*Metabolism* 8, 293 (1959)) have reviewed the metabolism of bone in various disease states (osteoporosis, osteomalacia, and osteitis fibrosa) using the concept of decreased bone density as an etiological approach in diagnosis. The evaluation of bone density was done by viewing the x-rays of the patient's bones.

In this review they discuss the various factors which may influence the calcification of bone, namely, inadequate intake of minerals, inadequate absorption, decreased transportation of nutrients to the bone by the body fluids, diseases of the bone itself (independent of extra skeletal causes), decreased rate of bone formation or increased rate of bone degradation, and renal defects causing increased excretion of calcium, phosphorus or protein. The bibliography of this review has 259 references.

### Botulism

Under strictly anaerobic conditions, *Clostridium botulinum* can produce in foodstuffs extremely potent neurotoxins. These toxins constitute one of the most potent poisons known ( $0.1 \times 10^{-5}$  micrograms is the LD<sub>50</sub> for the mouse). Recently Carl Lamanna (*Science* 130, 763 (1959)) has reviewed the present knowledge of the toxins responsible for botulism. Their chemical nature is discussed (proteins consisting only of amino acids), as are their toxic potency, their activity as oral poisons, mechanism of poisoning and the prophylaxis. This review has a good bibliography.

### American Board of Nutrition

The American Board of Nutrition will hold the next examinations for certification as a Specialist in Human Nutrition during

the week of April 11-15, 1960, in Chicago, Illinois. Candidates who wish to be considered for these examinations should forward applications to the Secretary's office not later than March 1. Application forms may be obtained from the Secretary, Robert E. Shank, Department of Preventive Medicine, Washington University School of Medicine, Euclid and Kingshighway, St. Louis, Missouri.

### Recent Books

*Principles of Nutrition*. Eva D. Wilson, Katherine H. Fisher and Mary E. Fuqua (Pennsylvania State University). Published by John Wiley & Sons, Inc., New York 16, N. Y. Pp. 460. Price \$5.95.

*Strontium 90 in Human Diet in the United Kingdom, 1958*. Report No. 1, Radiobiological Laboratory of the Agricultural Research Council. Her Majesty's Stationery Office, London; British Information Services, New York 20, N. Y. Pp. 75. Price 77¢ postpaid.

*Pork in Your Meals*. U. S. Department of Agriculture (Agricultural Research Service). Published by the U. S. Government Printing Office, Washington 25, D. C. 1959. Pp. 28.

*The Heinz Handbook of Nutrition*. Executive Editor, Benjamin T. Burton. Blakiston Division of McGraw-Hill Book Company, Inc. 1959. Pp. 439. Price \$5.75.

*U. S. Prospectus Research in Aging*. Veterans Administration. 1959. For sale by the Superintendent of Documents, Washington 25, D. C. Pp. 125. Price \$1.50.

*Symposium on Basic Research*. Sponsored by the National Academy of Sciences, the American Association for the Advancement of Science and the Alfred P. Sloan Foundation, May 14-16, 1959, New York City. Editor, Dael Wolfe. Published by the American Association for the Advancement of Science, Washington, D. C. Pp. 308. Price \$3.00.

## THE OPERATION OF A METABOLIC WARD

Occasionally it is valuable to review the origin and evolution of accepted practices in clinical investigation, just as it is well to take an occasional critical look at anything which has been accepted as common practice.

The development of balance studies in man under complete control in a metabolic ward is a feature of contemporary clinical investigation. The elaborate facilities we use today are in sharp contrast to those used in 1913 by Graham Lusk and Eugene DuBois or to the resources described by Walter Bauer and Joseph Aub (*J. Am. Dietet. Assn.* **3**, 106 (1927)). Yet these early research wards, designed to permit rigorously accurate collections and observations, were forerunners of the modern research ward, which is in effect a clinical laboratory. The development of research techniques has been related by E. C. Reifstein, F. Albright and S. L. Wells (*J. Clin. Endocrinol.* **5**, 367 (1945)).

Anyone with experience in the foibles of a well-run metabolic ward, the uniqueness of the human individual and the difficulty of using many subjects, is acutely aware of the impossibility of obtaining the necessary precision of diet, sample collection, observation and recording of signs and symptoms in even the best regulated general ward or private hospital room. The frustrations, complexities and confusion occurring at various times during the development of our metabolic ward have given us a background against which to review our current practices. Despite the most rigid and scrupulous attention to detail, errors and missed collections continue to pose vexing problems.

Periodically we evaluate our methods in the light of others' experience. Recently we

corresponded with the directors of metabolic research in 18 different medical centers, asking them to share with us their methods and experience. From 12 institutions we obtained detailed and comprehensive analyses. It was not surprising that all stressed a belief that an intelligently cooperative patient or subject was the primary requirement. It was agreed that this was more important than the special techniques of collecting and measuring specimens or using markers to identify time relations in dietary or experimental programs. Most agreed that metabolic studies conducted on service wards were fraught with a 50 per cent error.

Our own metabolic unit is constructed as follows:

### *Physical Facilities*

The metabolic unit has three large rooms, each capable of housing three patients in comfort. Across the hall are the nurses' office, an examining room, a utility room, a clinical laboratory and storage space. Nearby is a metabolic kitchen equipped to provide any type of diet or formula. A separate pair of bathrooms and toilets are equipped for collection of urine and feces. Refrigerators and freezers occupy the foyer outside these toilets. For diversion, a recreation room has tables and chairs for dining, a television set, games, crafts, books and magazines. Down the hall are the nutrition laboratories and the research laboratories of several clinical investigators.

### *Staff*

Because the facilities of the metabolic unit are available to all members of our clinical staff, the investigators change from time to time. A committee of four persons acts upon proposals for projects and assists



informally in the supervision and conduct of each study. Most studies are conducted by one or more members of this committee, whose main interests are nutrition, metabolism, endocrinology, cardiac disease and gastroenterology.

Nursing care of high caliber is provided by a staff of registered and practical nurses. All have been indoctrinated in the principles of investigative methods and the necessity for attention to minute details. They keep accurate records of daily activity, complaints, symptoms and behavior. Great emphasis has been placed on absolute honesty since errors occur despite scrupulous care. Such errors must be known.

Dietetic services provide more than the formulation and serving of prescribed diets, although these are important. Dietitians assist in planning all studies and conduct many of their own. They are skilled in performing many biochemical tests and are most helpful in evaluating and interpreting data.

The laboratories are staffed by technicians, graduate students and assistants, who learn to perform all manner of tests, some simple and routine, some elaborate and complex.

#### *Patient Material*

The people who serve as subjects on our metabolic ward are of three types. (1) Patients in the hospital who have a disease or condition of particular interest. (2) Healthy volunteers (usually medical or dietetic students) who may be studied as outpatients. (3) Healthy volunteers from a state penal institution who have been selected because of willingness, stability of personality and good health.

#### *Policies of Operation*

Often colleagues whose interests lie in other regions of medicine speak with disparaging words of a "metabolic study" as though it were slightly degrading. To them it may mean an attempt to reduce a fat patient or a routine testing of a new thera-

peutic agent. While either of these might be the subject of an investigation, it is essential that all persons participating in a metabolic study should understand the necessity for a program planned to the smallest detail. By studying the work of others and planning methods of his own, the erstwhile investigator either gains in enthusiasm or quits in despair.

A protocol must be prepared and reviewed informally by the committee. This policy allows us to dissuade the casual investigator as well as help the enthusiastic young clinician strive for excellence through sound planning and precise execution, allowing him to mature in an environment of friendly but critical assistance. By the same token, nurses learn details, methods and principles not taught in their usual curriculum, and dietetic students practice their skills while performing investigations of their own, usually in conjunction with a larger project.

The details of routine operation are simple and strict. Each patient is under the control and supervision of a physician who records a detailed history and physical examination, examines the patient daily, makes progress notes and writes orders for all medications and changes of routine. He also performs all venipunctures and special procedures.

The nurses, in addition to their routine duties of patient care, must observe the patients continuously. This reduces the risk of loss of specimens, eating of unauthorized food or any other disruption of the experimental program. They also collect, measure, record, label and preserve all specimens which are to be saved, and distribute aliquots of them to the appropriate laboratories. The dietitians furnish exactly measured food on attractive trays, or formulas disguised as well as possible. They participate in all phases of the study from planning, to chemical determinations, evaluation of data, and reporting of results.

All records are filed permanently in the metabolic ward. None is sent to the hospital record room. This policy requires some duplication of records when ill patients are

studied, but it has avoided the irksome irritation of the lost chart.

The last and most important aspect of operation deals with the subject or patient. Despite relentless effort and great expense, no successful study can be performed without enthusiastic and understanding cooperation from him. Careful selection and complete explanation and detailed instruction are necessary to insure any measure of success. The volunteer must be a free agent, but frequent encouragement and friendly reminders when mistakes occur serve to impress upon him the necessity for compliance and honesty in every action.

When confusion arises it almost invariably stems from lack of understanding. To reduce this the staff holds informal weekly conferences to discuss everything concerning the investigation. All routines, diets, medications or tests, and any changes are recorded in the chart, and each member of the team is notified. These measures, together with frequent discussions, help maintain smooth operation.

The cost of operating a metabolic unit such as ours is staggering. The average occupancy of between five and six patients daily throughout the year taxes the facilities of our staff and laboratories to the utmost. To calculate the daily cost per patient one must subdivide expenses into several categories. If one considers only room and board, the figure is approximately \$11.00 daily. But if one considers salaries, cost of equipment and operating laboratories and the many other items of expense, our cost is more than \$50.00 per bed per day.

Any patient assigned to our metabolic ward retains the status he had in the general hospital, as a private, semi-private or indigent patient. In the case of volunteers from a prison, the hospital administrators have generously provided routine facilities.

The financial support for the ward has been derived in large measure from grants in aid from the National Institutes of Health, The Nutrition Foundation, The National Vitamin Foundation and The American Cancer Society, supplemented by smaller grants from pharmaceutical companies and other sources.

Is such expense extravagant and unwarranted? Sometimes we wonder. Nevertheless, a metabolic ward is the only facility that can control the subject, provide a stable environment, a precise diet and accurate collection of specimens. Although the cost is great, the results in terms of reliable information justify the expense. Even diseases of the cardiovascular or gastrointestinal system must be studied by such methods if they are to be fully understood, and perhaps this applies to other diseases as well. More important, however, is the training of a corps of physicians, nurses, dietitians and technicians in rigorous methods which may yield important answers in the future.

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## VASCULAR DISEASE AND THE GROWTH, REPAIR, REGENERATION AND DEGENERATION OF VASCULAR ELASTIC MEMBRANES

*The vascular lesions resulting from remodelling or repair of the arterial walls and factors controlling local fibrin deposits are involved in degenerative arterial diseases.*

Several interesting aspects of the genesis of degenerative arterial diseases seem to arise from the recent studies in this field by T. Gillman and co-workers in South Africa.

These workers have repeatedly expressed opinions concerning the genesis of arterial diseases which, while incorporating the ideas expressed by several other workers, have also

added some new ideas and facts. They maintain that occlusive diseases are probably the result of two distinct but usually concurrent processes, *i.e.*, injury to the arterial walls themselves culminating in vascular sclerosis, and the intra-arterial thrombotic processes, probably occurring repeatedly, particularly at sites of previous vascular injuries and the scars resulting therefrom (*Lancet* II, 1117 (1957); II, 901 (1958)).

Pursuing the problems along these two lines, Gillman and co-workers have been analyzing, first, the basic structure of vascular elastic membranes, their mode of growth and reactions to injury, and second, the intravascular factors which might play important roles in promoting or retarding mural thrombotic or fibrin deposits over sites of previous injuries and the effects on these of fat intake and sex hormones.

Their studies of the structure and post-natal development of the vascular elastic membranes, their reactions in human and experimentally induced lesions indicate that arterial elastic membranes are not homogeneous in structure, *i.e.*, they seem not to be comprised solely of "pure" elastic tissue, in contrast to what is the apparent case for the ligamentum nuchae. They suggest that the vascular elastic membranes are comprised of "collagen-like" hyaline "cores" enclosed by an elastic-like component, this whole complex, in turn, being sleeved by an irregular coat of mucopolysaccharide. This opinion is based upon their extensive morphological and histochemical studies of the vascular elastic membranes in man and rats at different ages and in various pathological states (*A.M.A. Arch. Path.* 59, 733 (1955); 67, 624 (1959); *Connective Tissue*, p. 128. Blackwell, Oxford (1957); *J. Mt. Sinai Hosp.* 24, 857 (1957)).

Their recent analysis led these investigators to suggest that, in man, the vascular elastic membranes are remodelled during growth, an opinion to which they were initially led from studies of *Lathyrus*-intoxicated rats (see below). Such remodelling may depend upon a continuous interchange

of the molecular components of the various parts of the vascular elastic membranes, a process which may involve in some way the irregular perimembranous sleeves of mucopolysaccharide. They record that alterations in certain tinctorial reactions of elastic-like components of vascular elastic membranes, which occur spontaneously even in infants (perhaps as part of normal growth and remodelling) are almost invariably associated with increases in the mucopolysaccharide surrounding the areas of change (*A.M.A. Arch. Path.* 67, 624 (1959)).

These workers also noted a progressive series of associated changes in extracellular peri-membranous fat, in intimal cellularity, amount of mucopolysaccharide and in the staining of the affected vascular elastic membranes. These changes seem to culminate in marked intimal thickening and patchy sclerosis and so-called "reduplication" of the related vascular elastic membranes. They consider that "reduplicated" vascular elastic membranes are not comprised of "true" tissue but of what they call "pseudo-elastic" tissue (*A.M.A. Arch. Path.* 59, 733 (1955); 67, 624 (1959)).

The vascular elastic membranes seem to be replaced by regeneration during normal growth and aging of arteries. Such regeneration is distinguishable from repair by certain definable criteria. "Reduplication," or rather appearance of "pseudo-elastic" membranes seems to indicate some derangement in normal regenerative processes with resulting architectural disorder, *i.e.*, repair.

These investigators suggest that terminal sclerotic lesions in the arteries of young adults may represent the late end result of earlier deranged remodelling, which probably first occurs during the rapid growth of arteries at adolescence or even during infancy. The sclerosis of arteries in aged individuals, on the other hand, may reflect the gradual deterioration of the individual's capacity to regenerate the wear and tear changes normally occurring in all arteries. Repair processes then ensue with unavoid-

able and progressive scarring as the outcome (*Lancet* II, 1117 (1957); II, 901 (1958)).

This approach may be of some value in studying, for example, the arterial changes in diabetics. For it is now recognized that diabetes must be present for some years before arterial and capillary pathology become manifest. Could the overall disturbances in carbohydrate metabolism in long-standing diabetes find some expression also in altered peri-membranous and other connective tissue carbohydrate turnover (such as is in the mucopolysaccharide sleeves), thus leading to the pathological changes characteristic of advanced diabetes? This possibility is supported by H. Grossfield's recent analysis of the role of carbohydrate metabolism in maintaining the structural proteins in arteries (*Nature* 184, 38 (1959)).

Gillman and associates suggest that the repair of vascular elastic membranes, injured acutely by single short episodes of calciferol intoxication in rats, closely simulates that observed in healing cutaneous injuries. Their data indicate that such arterial injuries are, however, repaired only slowly and that even single episodes of acute arterial injury, if severe enough, may culminate only months later in rats (and perhaps only years later in man) in vascular scarring, *i.e.*, sclerosis. Thus they suggest that some forms of arteriosclerosis in man, especially in certain arteries (*e.g.*, coronaries) may simply be the late end result of single episodes of acute arterial injuries incurred traumatically or metabolically.

Furthermore, their studies of *Lathyrus*-intoxicated rats led them to suggest that the arteries of the rat are most susceptible to the toxic action of nitriles at a time when these arteries are growing most rapidly in length and diameter (and therefore being remodelled), particularly during the first few weeks post-natally or the last week of intra-uterine life (*J. Embryol. Exp. Morph.* 6, 270 (1958); *Schweiz. Z. Path. Bakt.* 22, 62 (1959)). The way in which such vascular growth, which must be occurring at these times, is achieved has still not been divulged,

but it may perhaps be accompanied by a process comparable to that seen in long bones, *i.e.*, by endarterial (endosteal) lysis, closely coupled with intramedial or adventitial (periosteal) neogenesis of arterial membranes (bone).

In the view of these investigators, the aortic ruptures in *Lathyrus* intoxication may be due, not so much to increased lysis, but rather to depressed neogenesis of vascular elastic membranes, an opinion supported by recent observations that collagen synthesis is depressed by nitrile intoxication (*Schweiz. Z. Path. Bakt., loc. cit.*). Such alterations in the synthesis of "collagen-like" components of vascular elastic membranes may involve some block either in the synthesis of the pro-collagen or, alternatively, in the linking of the submicroscopic units of collagen fibrils.

Their work thus provides a further stimulant for the re-examination of the architecture of vascular elastic membranes, especially by the use of electron microscopy. Analysis of the mechanism of regeneration of these vascular elastic membranes, and especially of their remodelling as the arteries grow during adolescence, seems particularly worthy of study with radioactive isotopes. A similar approach to the study of regeneration and repair of vascular elastic membranes in various diseases, *e.g.*, in long-standing diabetes, may also provide data of great value for our understanding of degenerative arterial diseases.

These investigators also suggest that the earliest injuries to human arteries may be initiated most readily when the vessels are growing and being rapidly remodelled, especially during adolescence. They propose that the deterioration of such early initial arterial injuries into frank vascular scars probably takes years. R. L. Holman *et al.* (*Am. J. Path.* 34, 209 (1958)) have recently shown that endarterial fatty streaks appear most frequently during adolescence, while intimal scars develop only ten to 15 years later.

Such deterioration of early injuries to

## FASTING AND ELECTROLYTES (ANOREXIA NERVOSA)

*Patients with anorexia nervosa show a low serum potassium level (especially with excessive weight loss) accompanied by reduced levels of sodium and chloride and elevated levels of carbon dioxide. High intakes of potassium overcome these abnormalities.*

Anorexia nervosa is a relatively rare disease occurring predominantly in women between the ages of 15 and 40 years (*Nutrition Reviews* 11, 271 (1953)). The individuals afflicted with this disease frequently starve themselves to death. There is at present no satisfactory etiological explanation for the condition. Psychiatric treatment has been the only therapy which has shown any success, but complete recovery has occurred in only a small percentage of the cases.

J. R. Elkinton and E. J. Huth (*Metabolism* 8, 376 (1959)) at the University of Pennsylvania recently reported on the disturbances in electrolyte metabolism in patients with anorexia nervosa. They stated that since 1938 only 20 cases of anorexia nervosa had been seen at the University Hospital. For these patients, Elkinton and Huth plotted the total carbon dioxide content of the serum against the per cent of body weight loss. They found that the values were within normal limits except when the weight loss exceeded 25 per cent, in which case the level of carbon dioxide was higher than normal. When similar graphs were made for the serum levels of chloride, sodium and potassium, these electrolytes were also within normal limits except when the body weight loss exceeded 25 per cent. In the latter situation the levels of the electrolytes were below normal.

To establish the nature and the mechanisms responsible for the alterations in electrolyte concentrations, Elkinton and Huth kept two patients with anorexia nervosa in a metabolic ward. One patient was 34 years old and weighed 35.5 kg. (56 per cent of her initial weight), and the other was 43 years old and weighed 32.6 kg. (65 per cent of her initial weight). Each patient was given a standard, neutral ash diet which

provided about 30 mEq (0.12 g.) of potassium per day. On this basal diet, there was a retention of 23 mEq of potassium. When the patients were given increasing amounts of potassium (as the chloride), they retained most of it even at intake levels of 160 mEq (6.2 g.).

During the period of potassium administration, the serum potassium levels increased from 2.5 mEq per liter to normal values of 3.6 to 4.4 and there was a reduction to normal of the serum carbon dioxide content. However, there was no essential change in the nitrogen balance. Potassium was retained in excess of nitrogen, with potassium to nitrogen ratios as high as 15.1 compared to the normal range of 2.4 to 3.0.

The retention of potassium was associated with the retention of chloride as shown by the increases in the chloride space, ranging from 2.5 liters to 11.9 liters in the different experimental periods. These changes were far greater than the changes in body weight ( $-0.68$  to  $+2.10$  kg.).

The above observations suggested to Elkinton and Huth that there was a higher concentration of chloride in some body fluid other than plasma. However, they pointed out that this suggestion is open to question since their subjects may have surreptitiously discarded their vomitus and thus vitiated the calculated chloride balance. Individuals with anorexia nervosa show a high tendency to vomit and are reluctant to acknowledge this fact (Elkinton and Huth, *loc. cit.*).

The hyponatremia originally present in both patients was corrected during the period of positive potassium balance, and this correction in sodium balance was achieved in the absence of an increased sodium intake.

The marked hypochloremia alkalosis and hypokalemia seen in the two anorexia nervosa patients may very well have been the

result of vomiting, since kidney and adrenal functions appeared to be normal. The normal adrenal activity was shown by a normal 24-hour urinary excretion of aldosterone and other alpha-ketolic steroids. Additional evidence for normal adrenal activity was provided by J. M. Berkman, C. A. Owen, Jr., and T. B. Magath (*Postgrad. Med.* **12**, 407 (1952)), who studied a 36-year-old woman whose height was 156.2 cm. and whose weight was 26.3 kg. Her 24-hour urine sample contained 1.4 mg. of 17-ketosteroids and 0.42 mg. of corticosteroids. Both of these values were normal.

Hypokalemia as severe as that seen in the above patients was not observed in any of the ten patients with anorexia nervosa studied by H. Ljunggren, D. Ikkos and R. Luft (*Acta Endocrinol.* **25**, 290 (1957)). Two of their patients had plasma potassium

values of 2.9 and 3.0 mEq per liter as well as low plasma sodium levels. One had low chloride levels.

The above studies on patients with anorexia nervosa emphasize the marked differences that are likely to occur in the electrolyte balances, even when these patients are kept in a metabolic ward. Part of this probably stems from the difficulty in securing complete cooperation due to the disturbed psychological state of these patients. Their tendency to surreptitiously discard their vomitus poses a possible loophole which should be carefully checked if metabolic studies are to be carried out.

The outstanding characteristic of the electrolyte disturbances in these patients is the tendency for a low level of serum potassium to develop as they lose more than 25 per cent of their initial body weight.

## SYNDROMES OF MAGNESIUM DEPLETION AND RETENTION IN MAN, PART I.

*A syndrome of magnesium deficiency probably can be defined in certain patients with alcoholism or electrolyte imbalance. This consists of sudden psychoses, neuromuscular hyperirritability and electrocardiographic changes.*

Although the body contains about 21 g. of magnesium (11 g. in the skeleton and 9.5 g. in the cells) only 0.5 g. is found in extracellular water, about 2 mEq per liter of plasma. Our knowledge of magnesium distribution and metabolism is much less than of many other electrolytes, no doubt because it is such an insignificant part of the extracellular fluid electrolyte total and because until recently its measurement has been tedious. Nevertheless it is the second most abundant intracellular cation, comprising about 6 to 20 mEq per liter compared to about 120 mEq per liter for potassium. Renal conservation is excellent and, as with other electrolytes, the plasma level probably bears little relation to the intracellular concentration, so that it is not surprising that syndromes attributable to changes in magne-

sium have been poorly correlated with its plasma concentration.

An excess of magnesium given parenterally leads to narcosis, which is completely reversed within seconds after sufficient calcium salt is given intravenously. Magnesium is important in neuromuscular conduction of skeletal and cardiac muscle; it activates numerous enzymes involved in carbohydrate metabolism and is required for the action of a variety of other intracellular enzymes. It has long been known as a component of chlorophyll, in whose structure it holds the same position that iron does in heme.

Increased serum magnesium levels have been observed in acute renal failure by W. E. C. Wacker and B. L. Vallee, using a multichannel flame spectrometer (*New Engl. J. Med.* **257**, 1254 (1957)). They point out

the similarity of symptoms of uremia and of hypermagnesemia and suggest that the latter may account for some of the weakness and somnolence of the former. Since the electrocardiographic and clinical signs of hyperkalemia and hypermagnesemia are also similar, the authors thought that the latter might occur in acute renal failure and be responsible in part for the symptoms of "potassium intoxication." Indeed, some cases of this syndrome, with little increase in serum potassium, did prove to have hypermagnesemia. In general, however, increases of serum magnesium roughly paralleled those of potassium.

A deficiency syndrome has been experimentally studied in dogs and rats, which develop vasodilatation, cardiac arrhythmia, hyperirritability, spasticity, convulsions and death (*Nutrition Reviews* 17, 112 (1959)). Although some of the physiologic and biochemical actions of this element are well known, a magnesium deficiency syndrome in man has not been clearly defined and clinically recognized in the past.

The syndrome of "grass staggers" or grass tetany of cattle is associated with a sudden decrease in serum magnesium concentration and is partially preventable by fertilization of grassland soil with magnesium (*Nutrition Reviews* 12, 214 (1954)). Since the grass is not magnesium-deficient in the first place, the syndrome does not represent a simple deficiency. Likewise magnesium deficiency develops in calves fed too long on milk, apparently because of a progressively lesser utilization of dietary magnesium (*Ibid.* 13, 12 (1955); 16, 173 (1958)).

Other aspects of magnesium metabolism have been reviewed in this journal (*Nutrition Reviews* 12, 77, 181 (1954); 15, 237 (1957)). Wacker and Vallee have published a comprehensive review with 232 references (*New Engl. J. Med.* 259, 431, 475 (1958)).

E. B. Flink *et al.* have published an article entitled "Evidences for Clinical Magnesium Deficiency" (*Ann. Int. Med.* 47, 956 (1957)). They describe "a clinical syndrome charac-

terized by muscle tremor, twitching and more bizarre movements, occasionally by convulsions and often by delirium," which they attribute to magnesium deficiency. They believe that deficiency of this element is a not uncommon clinical problem with "distinctive clinical features."

The authors studied "a large group of patients with chronic alcoholism and tremulousness and a few postoperative patients and patients with pyloric obstruction and alkalosis." They measured serum and urine magnesium levels and obtained partial balance data in some. Results are reported in 29 patients with tremor and delirium (mean serum magnesium  $1.58 \pm 0.3$  mEq per liter), and in another group of 21 patients with tremor and muscular twitching without delirium (mean serum magnesium  $1.88 \pm 0.4$  mEq per liter). The authors' previous measurements on "normal subjects and patients without neurologic manifestations" gave a mean value of  $227 \pm 0.26$  mEq per liter. Four illustrative cases are reported, and partial balance studies in three others.

*Case one*, a 70-year-old man, had a ureteral transplantation into the ileum followed for 12 days by continuous suction drainage of gastroduodenal contents. "Adequate amounts of potassium and sodium salts were given." Fever and abdominal distention developed on the seventh postoperative day, together with confusion, belligerence, athetoid and choreiform movements, tremor and muscle twitching. On the eleventh day, the serum magnesium concentration was low (1.46 mEq per liter). When a total of 16 g. of magnesium sulfate was given intramuscularly over three days, the symptoms disappeared within 36 hours. No explanation is given for the serum albumin of 2.0 g. per cent and globulin of 3.6; one wonders if this patient had cirrhosis of the liver.

*Case two*, a 59-year-old man with decompensated cirrhosis, developed incontinence, confusion, noisiness, restlessness, disorientation, tremor and twitching, progressing to delirium and semi-coma. The manifestations



were relieved 24 hours after a course of magnesium sulfate was begun. The diagnosis of hepatic coma is not discussed.

*Case three*, a 48-year-old man, had been vomiting for a week, presumably from pyloric obstruction due to an ulcer of the duodenum. He had severe signs of tetany; a convulsion occurred after intravenous calcium gluconate injection, and severe delirium appeared by the fourth day. Magnesium sulfate was given intramuscularly from the fifth to the fourteenth day. The sensorium cleared considerably on the seventh day, 36 hours after the start of magnesium treatment, and became completely clear on the eighth day. This man had severe alkalosis when he came to the hospital, with decreased serum potassium level, extreme diminution of serum chlorides and a high blood urea nitrogen. Undoubtedly his electrolyte pattern was greatly altered in other ways not measured.

*Case four*, a confused and hallucinating alcoholic man of 34 years, had a severe hypopotassemic alkalosis. The authors point out that delirium worsened after potassium was given, and tremor and other neuromuscular signs appeared. These improved eight hours after magnesium therapy was started and cleared entirely in 46 hours. The reader is not told the cause of this patient's alkalosis, nor whether he had cirrhosis. Vomiting would scarcely explain the findings; one presumes that the patient was thought to have delirium tremens.

The authors point out that "the development of neuromuscular manifestations in cases three and four when hypopotassemia was being corrected . . . may well be related to the experimental observation that potassium accentuates the manifestations of magnesium deficiency."

Flink and his colleagues studied the urine excretion of a parenteral dose of magnesium sulfate in three patients with chronic alcoholism, tremor, twitching and mild delirium. They found 35 to 67 per cent in the urine, whereas the normal subject excretes all of

the dose in the urine under comparable circumstances.

A number of criticisms may be raised. The observations suggest a clinical syndrome of magnesium deficiency, but lack the dramatic cure one observes with specific magnesium replacement therapy in experimental animals. To the reader, the syndromes of hepatic coma, delirium tremens, hypopotassemia and magnesium deficiency appear insufficiently distinguishable with the evidence at hand. This is not to deny that the patients may have had magnesium deficiency; probably the best evidence that they did is the retention of the element after a parenteral dose.

Magnesium deficiency has been produced experimentally in man by M. G. Fitzgerald and P. Fourman (*Clin. Sci.* **15**, 635 (1956); *Nutrition Reviews* **15**, 232 (1957)), but it is difficult to achieve. Reduction of intake even to 1.1 mEq per day was not enough to cause a large negative balance, so a cation exchange resin was added to the deficient diet of one subject. Even so, the two subjects attained total negative balances of only 46 and 72 mEq respectively in 20 and 24 days (less than 5 per cent of the body's total of approximately 2000 mEq). No change was detected in blood pressure, electrocardiograms, ability to concentrate the urine, or serum magnesium level, even though the urinary excretion after the twelfth day was reduced to 1 mEq per 24 hours.

With large body stores, the availability of bone magnesium to replace tissue losses, and efficient renal conservation of the element, it is clear that the cellular content can be very well protected. Nevertheless, at the end of the study 25 and 42 per cent of intravenous doses of magnesium salts were retained in the two depleted subjects, whereas none was retained in the controls.

J. W. Agna and R. E. Goldsmith have reported three cases of primary hyperparathyroidism with "clinical abnormalities believed to be related to magnesium de-

iciency" (*New Engl. J. Med.* **258**, 222 (1958)). All had neuromuscular irritability; in two this subsided after magnesium sulfate therapy. The authors conclude that "the clue to the presence of hypomagnesemia may be the occurrence of tetany, after surgical correction of hyperparathyroidism, that is refractory to the administration of large amounts of calcium." Whether the hypomagnesemia is a primary effect of parathormone or a secondary result from skeletal or soft tissue depletion is not apparent.

Flink and his colleagues have attempted a very difficult task; to define a clinically recognizable syndrome of magnesium deficiency when even its laboratory detection is a matter of inference. The manifestations which the authors "have ascribed to magnesium deficiency in patients can be listed

as follows: muscular twitching and tremor . . . choreiform and athetoid movements . . . rarely, carpopedal spasm and positive Chvostek's and Trousseau's signs, profuse sweating; tachycardia and occasionally, fever; mild anxiety to severe agitation; mild to severe delirium with hallucinations, confusion and disorientation, abusive language and maniacal behavior; finally, convulsions and coma."

Surely a number of etiologically disparate entities share various of these signs. The authors recognize that there may be little correlation between the serum magnesium concentration and the presence of signs or symptoms since serum electrolyte levels are a very poor measure of intracellular concentrations or body stores. This is borne out repeatedly in clinical practice.

### ENERGY METABOLISM IN MALNOURISHED INFANTS

*Metabolic rates in malnourished infants may be increased per unit of weight because of fat losses. Severe starvation will depress metabolic rate in infants as well as in adults.*

After nearly 30 years, the oxygen consumption of malnourished infants has been reinvestigated by F. Varga (*Pediatrics* **23**, 1085 (1959)). Studies made in the 1920's suggested that a high metabolic rate occurred in infant malnutrition in contradistinction to more recent studies in adults which showed a decreased oxygen consumption on starvation regimes (A. Keys *et al.*, *The Biology of Human Starvation*, University of Minnesota Press, 1950).

Varga reinvestigated this problem in eight infants with pyloric stenosis. By making observations when the infants were both starved and dehydrated, then following hydration, and later during the course of realimentation following specific corrective surgery, the influences of dehydration, starvation and feeding could be determined separately. Since infection did not play a role in the development of starvation in these infants, it had no bearing on their increased oxygen consumption.

While the normal value for oxygen consumption of infants in this age group is 7.1 ml. per kg. per minute, the average oxygen consumption of these infants was 4.9 ml. per kg. per minute. During the first three days postoperatively when the caloric intake began to reach minimal maintenance values of 70 calories per kg. and hydration was normal, the oxygen consumption dropped slightly to 4.6 ml. per kg. per minute. After the fifth postoperative day when the intake of food had returned to normal or above, values of 8.0 ml. per kg. per minute were found. This high oxygen consumption was thought to be due to the loss of fat which had occurred during starvation.

Studies of chronic malnutrition likewise revealed an increased oxygen consumption of 8.2 ml. per kg. per minute, but with a fall to 5.5 ml. per kg. per minute in the presence of semi-starvation.

The above studies suggested an explana-

In none of the groups did the administration of D-sorbitol alone for four weeks result in an increase in serum vitamin B<sub>12</sub>. In fact, on the average, there was a decrease. Further treatment of one group with sorbitol alone for another five weeks resulted in a small but insignificant rise. Treatment of the other two groups with sorbitol and vitamin B<sub>12</sub> caused a considerably greater rise in serum vitamin B<sub>12</sub> concentration. When the treatment was stopped, the serum vitamin B<sub>12</sub> values fell to within the normal range in one week.

The authors emphasize that variability within groups of normal subjects and inherent variability in the assay method make interpretation difficult. However they conclude that D-sorbitol does not act by stimulating the production of vitamin B<sub>12</sub> by intestinal microorganisms but rather that absorption of the vitamin is facilitated.

It is unfortunate that in no period in this experiment did the subjects receive vitamin B<sub>12</sub> alone. This would have provided an opportunity to measure the effect of D-sorbitol and vitamin B<sub>12</sub> separately as well as together, and would have provided a more controlled experiment. Also, there is enough of a trend toward higher serum vitamin B<sub>12</sub> concentrations after the administration of D-sorbitol alone to warrant a longer examination of this effect and to determine whether D-sorbitol administered with cobalt might not be more effective than D-sorbitol alone.

The second paper by Herbert *et al.* describes a study of the influence of sorbitol on vitamin B<sub>12</sub> absorption by subjects able to produce intrinsic factor. They used the Schilling test, measuring urinary Co<sup>58</sup> excretion for 24 hours after the administration of a test dose of labeled vitamin B<sub>12</sub>. They observed that when supraphysiologic doses of vitamin B<sub>12</sub> (30 micrograms per day) were administered, there was evidence of improved absorption as the result of the concomitant administration of about 10 g. of D-sorbitol.

When they ran similar tests on 12 subjects

who received only 2 micrograms of vitamin B<sub>12</sub> daily, the enhancing effect of D-sorbitol was less clear; although some positive effect was seen in eight of the 12 subjects.

The third paper by Chow *et al.* describes a study of the influence of D-sorbitol on vitamin B<sub>12</sub> absorption by pernicious anemia patients. They also measured absorption by following urinary excretion of radioactivity for 24 hours after administration of a test dose of 2 micrograms of Co<sup>60</sup>-labeled vitamin B<sub>12</sub>. A study of more than 100 healthy subjects showed that the average normal urinary excretion is about 11.0 per cent of the test dose. Twelve pernicious anemia patients, on the other hand, excreted less than 1.5 per cent of the administered dose in the urine, and most of them 0.5 per cent or less. The administration of 10 g. of D-sorbitol with the test dose of vitamin B<sub>12</sub> increased the absorption significantly in only one of the 12 subjects, but only into the low normal range.

In ten subjects with achlorhydria the administration of D-sorbitol did result in a small but consistent increase in the absorption of vitamin B<sub>12</sub>. Only 1.2 per cent of a test dose of 50 micrograms of vitamin B<sub>12</sub> was excreted in the urine when the subjects were given the vitamin alone. This was increased to 2.2 per cent by the concomitant administration of D-sorbitol. The increase was statistically significant but the values were still well below those for healthy persons.

One question about this study cannot be overlooked. Why did the authors use a test dose of only 2 micrograms of vitamin B<sub>12</sub> in their study of pernicious anemia patients and 50 micrograms when testing patients with achlorhydria? The question is of some pertinence in view of the results of Herbert *et al.*, who could not demonstrate a consistent effect of D-sorbitol on the absorption of a 2 microgram test dose of vitamin B<sub>12</sub> in normal subjects, whereas they could when the test dose was 30 micrograms.

Nevertheless, there is sufficient evidence

to indicate that D-sorbitol is ineffective as a therapeutic measure in pernicious anemia patients (Chow *et al.*, *Am. J. Clin. Nutrition* **4**, 434 (1956); **7**, 325 (1959); Ellenbogen *et al.*, *loc. cit.*; Herbert, *Am. J. Clin. Nutrition* **6**, 547 (1958)).

It would certainly be of interest, however, to follow up these experiments with studies

in which various levels of labeled vitamin B<sub>12</sub> and various levels of D-sorbitol are administered to the occasional pernicious anemia patient who appears to respond to treatment with D-sorbitol. Also, a methodical study of the effect of different levels of D-sorbitol on vitamin B<sub>12</sub> absorption in normal subjects would be valuable.

## CARDIAC PATIENTS IN UNDERDEVELOPED COUNTRIES

*Cor pulmonale is more common in India than in the United States while the reverse is true for coronary atherosclerosis. Life expectancy in the United States is more than double that of India.*

While in the West we ponder the evils of cholesterol in the blood stream or the deplorable incidence of obesity, the greater half of the world's population hungers for more food. It has become popular to attribute our high incidence of coronary vascular disease largely to our high standard of living, our abundance of meat, egg and milk products. Perhaps we can profit by comparing our own situation with that existing in India, a land of dense population, incredibly impoverished, yet striving to correct the condition which hampers its progress.

Starvation as a clinical syndrome is a curiosity in the United States, since it has all but vanished. We have read of it in Japanese prison camps (*Nutrition Reviews* **5**, 86 (1947)), and in persons subjected to famine for other reasons (*Ibid.* **6**, 210 (1948); **7**, 250 (1949)). H. L. Taylor and A. Keys studied a group of volunteers who were starved until they lost 24 per cent of their body weight (*Science* **112**, 215 (1950)), and reported that their metabolic rate was decreased 31 per cent and their cardiac work by half. Moreover, they developed an apathy and physical lethargy which was nearly incapacitating.

Malnutrition is a state of anxiety, hunger and exposure, constantly aggravated by inadequate food and relentless infection, which must lead to disillusionment and despair of a degree unknown to us.

With this in mind, the studies by S.

Padmavati of patients with heart disease in India are of interest (*Am. Heart J.* **58**, 418 (1959)). She calls attention to the apathy which made collection of accurate data so difficult and perhaps inaccurate. However, she was able to obtain figures from a few sources and to evaluate them with proper regard for their significance. The first set of data came from established hospitals in some of the 16 states of India; the second from a survey of selected groups (reliable but incomplete), and the third from records of medical insurance agencies which serve selected social groups.

Comparison of the incidence of deaths from heart disease in hospitals, which was 3 per cent for India and 50 per cent for Western countries, demonstrated the fallacy of statistics. Surely this implies that cardiac patients in India cannot be hospitalized but must live and die in their own communities. For this reason, Padmavati suspected that the incidence of heart disease in India, reported at 1 to 3.6 per cent, was grossly underestimated.

However, she was able to make some valid comparisons for certain groups of cardiac patients. The incidence of various types of heart disease in Delhi from 1951 to 1955 as compared with that in the United States was as follows:

Rheumatic heart disease (39.1 per cent vs. 23.5 per cent for New England) was the commonest form of cardiac disorder in

Delhi and reached its greatest incidence between the ages of 20 and 30 years. The author felt that this age distribution was more apparent than real, since patients did not seek medical examination until symptoms became troublesome. Consequently the disorder was not recognized until physical exertion (manual labor in men, pregnancy and delivery in women) precipitated congestive failure.

Although hypertensive cardiovascular disease was slightly less common in India than in the United States (21.1 per cent *vs.* 26.2 per cent for New England), it constituted the second major form of heart disease. Perhaps it is significant that it followed toxemic pregnancies in 35 per cent of the cases.

Cor pulmonale, the third most common form of heart disease in India (16.6 per cent *vs.* 1.1 per cent for New England), afflicted men and women with equal frequency. The duration of symptoms before death was short, and autopsy disclosed advanced emphysema (not tuberculosis) as the principal cause. The author favored the theory that respiratory infections were prevalent and remained untreated due to conditions of poverty and insufficient numbers of physicians.

As might be anticipated in a country whose food supplies include relatively little animal fat and animal protein, and whose populace seldom achieves advanced age, the incidence of coronary atherosclerosis was low (11.3 per cent *vs.* 48.5 per cent for New England). Measurements of the concentration of blood cholesterol revealed the expected lower values, particularly in the poorer socioeconomic groups.

Padmavati concluded that much of the cardiac disease of India was a result of inadequate facilities, insufficient numbers of trained physicians, and meager supplies of drugs and antibiotics. In 1951 the life expectancy of an Indian was 32 years as compared with approximately 68 years in the United States.

However, she did not mention several other factors which might be of equal or greater importance. Inadequate supplies of food, presumably of poor nutritional value, could account for early deaths and the high incidence of infection. Experience in the United States has established the fact that inadequate dietary protein for mothers and children is accompanied by increased rates of toxemic pregnancy and of rheumatic fever in children. Susceptibility to infection is so closely entwined with malnutrition and exposure to the elements, that the combined effect leads to illness and early death.

Although Padmavati intended to portray the inadequacies of medical facilities in her country as the major factor in the production of cardiac disease, she simultaneously supplied evidence of the deleterious effects of inadequate nutrition upon the heart. Those of us who concern ourselves with the alarming incidence of atherosclerotic heart disease in this country might profit from this article. It is evident that India, while suffering little from coronary disease, has a much greater burden to bear, since many of its people do not live to an age at which coronary disease would occur. Meanwhile the factor of "survival of the fittest" has been partially eliminated from the Western scene so that more persons have become eligible for this disorder.

## FLUORIDE IN METABOLISM

*Kidney changes, including lowered concentrations of fatty acid oxidase, may be of sufficient importance to account for many of the effects observed in fluorotic animals.*

Although it is known that two- or three-fold increases in protoplasmic fluoride con-

centrations may be toxic to the actively metabolizing cells of the animal body, the

exact locus of the physiological action of fluoride is not known. Most studies of fluoride have been concerned with its effect upon the skeleton and teeth and its beneficial effect in preventing dental decay, but as early as the turn of the century J. H. Kastle and A. S. Loevenhart (*Am. Chem. J.*, **24**, 491 (1900)) reported that fluoride inhibited lipase.

R. B. Johnson and H. A. Lardy (*J. Biol. Chem.* **184**, 235 (1950)) later demonstrated that fatty acid oxidase activity was inhibited by 0.01 molar fluoride. The closely related acetate activating system was inhibited by 0.0001 to 0.0005 molar fluoride *in vitro* (A. C. Aisenberg and V. R. Potter, *Ibid.* **215**, 737 (1955)). This is within the concentration present in soft tissues under conditions of fluorosis. R. F. Miller and P. H. Phillips (*J. Nutrition* **56**, 447 (1955)) found that the toxicity of fluoride was enhanced by high levels of dietary fat. This effect was independent of the chain length of the constituent fatty acids.

In a recent study, A. H. Sievert and Phillips (*J. Nutrition* **68**, 109 (1959)) have examined the *in vivo* effects of fluoride upon fatty acid oxidase reactions, enhancement of fluoride toxicity by dietary fat and the acetylation capacity of tissues in fluorosis.

Weanling rats of the Holtzman strain, weighing between 40 and 45 g. were fed semi-purified diets into which fluoride was incorporated as sodium fluoride. All diets containing increased fat levels were made isocaloric with the normal fat diets by the substitution of roughage for an equivalent of sucrose. At the close of each experiment the animals were sacrificed and the tissues needed were quickly excised, chilled and homogenized in isotonic sucrose. Mitochondria were suspended in isotonic sucrose so that the fatty acid oxidase activity could be determined with the Warburg apparatus.

The effects of two levels of fat and two levels of sodium fluoride upon fatty acid oxidase activity in the female rat were studied after the diets had been fed for six

to eight weeks. Growth rates were definitely retarded by dietary sodium fluoride and were further depressed by high dietary fat in the fluorotic rat. However, the mitochondrial fatty acid oxidase activity of the livers of these fluorotic rats and of their controls were essentially the same; hence there was evidence of a direct effect of fluoride on this enzyme system. Moreover, the high-fat diet had no influence upon the fatty acid oxidase values, despite its effect of retardation of growth.

G. R. Drysdale and Lardy (*J. Biol. Chem.* **202**, 119 (1953)) have reported that, although fluoride inhibition of fatty acid oxidation was negligible in a soluble system prepared from rat liver mitochondria, it was complete in a kidney cyclophorase system. The authors point out that since in liver the end product of the oxidation is mainly acetoacetate and since kidney and other organs further metabolize acetoacetate to carbon dioxide and water, the locus of the inhibitory action of fluoride in the fatty acid oxidase system may have occurred at a step beyond the formation of acetoacetate.

The authors, therefore, undertook an experiment to test the effect of sodium fluoride feeding upon kidney fatty acid oxidase. A significant decrease in fatty acid oxidase activity was observable as early as the fourth to fifth day after the beginning of fluoride ingestion. Gross inspection of the kidneys at the four to five day interval indicated that they were unaffected, but chemical analysis showed a highly significant increase in kidney water content. After two weeks on the regimen, the kidneys from the treated animals were yellowish in color, edematous, and presented a granular appearance. Chemical analyses revealed striking decreases in fat and nitrogen concentrations.

The authors are of the opinion that these data did not define the sequence between the enzymatic, chemical and anatomical changes resulting from elevated fluoride ingestion. For example, the striking decrease

in fatty acid oxidase activity could not be accounted for on the basis of the decreased mitochondrial nitrogen content observed in the fluorotic rat kidney.

Since the high fat diet (15 per cent) was also without effect on the liver fatty acid oxidase system, the authors undertook to determine the mode of action of the fat in inhibition of growth. Analysis of fecal materials showed that the fat excretion in fluorotic rats was twice as great as in control rats. Similarly, the nitrogen content per gram of feces was higher in fluorotic rats. Fluorosis in the rat is accompanied by semi-starvation food intakes, but comparison with pair-fed control rats indicated that there was better utilization of the food taken in by the control rats. These results make it evident that it was the fluorosis per se, and not the starvation accompanying it, which was responsible for the altered excretion patterns.

Neither the cause nor the origin of the elevated fecal fat and dry matter observed in fluorotic rats was determined. However, the authors point out that lipid metabolism

is intimately associated with coenzyme A and acetyl coenzyme A. One of the functions of coenzyme A in the body is the transfer of acetyl groups. The effect of fluorosis on acetyl transfer was therefore tested, but in none of these experiments was any consistent difference noted in the per cent acetylation of para-aminobenzoic acid by fluoride-fed rats and their pair-fed controls. There was no evidence that the ability of the intact rat to acetylate aromatic amines was inhibited by dietary fluoride.

The authors conclude that the kidney changes, including lowered concentrations of fatty acid oxidase, lipids and nitrogen, may be of sufficient importance and magnitude to account for many of the effects observed in fluorotic animals. The decrease in kidney fatty acid oxidase activity, whatever its cause, must seriously interfere with the animal's ability to metabolize fat.

It should be emphasized that these interesting results on tissues from fluorotic animals have nothing to do with the favorable effects of minute concentrations of fluoride in lessening dental decay.

## CHEMISTRY OF INFLAMMATION AND REPAIR

*Chemical analysis of skin, injured by croton oil injections, for nitrogen, hexosamine and hydroxyproline were correlated with the histological changes in the same tissue.*

The histological description of tissue inflammation and its repair have been known for some time. With the development of chemical procedures, studies have been undertaken to correlate the changes seen on histological examination with the chemical alteration going on within the tissue. Recently, R. Jacob and J. C. Houck (*Surg. Gynec. Obstet.* **109**, 85 (1959)) have studied the chemical events induced by a croton oil inflammation. Using semi-micro procedures, these investigators determined the nitrogen, hexosamine and hydroxyproline content of the skin of animals before and following the production of a croton oil induced inflammation. The analysis of these

three substances, the authors believed, would give them some index of the granulation tissue formation (hexosamine), collagen (hydroxyproline) and tissue protein (nitrogen) in the skin.

For this study 60 male rats weighing between 220 and 250 g. were used. The animals received intradermally 0.4 ml. of croton oil in the right dorsal area. A corresponding area on the other side of the body was used as a control. At various times ranging from three to 21 days following injury, groups of six animals were sacrificed and both the injured and uninjured areas were studied for histological and chemical changes. There was usually a necrotic re-



sponse to the croton oil within 24 hours after injection, and the ulcerous lesion covered an area of about 1.5 sq. cm. Seven days later an eschar formed which thickened during the following days and sloughed on the average of 17 days post-injury. Twenty-four days after the injury, the wound was healed and overgrown with hair.

To negate the influence which variations in the water and fat content of the skin would have upon the chemical content of the various substances studied, these investigators expressed all values as micromoles of hexosamine or hydroxyproline per millimole of nitrogen. Expressed in this manner, there appears to be no statistical difference in samples obtained from various areas of the skin.

In the injured areas of skin, it was found that there was a marked decrease in the ratio of hydroxyproline to nitrogen beginning about the third day following injury. The levels then remained constant until about the eighteenth or nineteenth day post-injury when the hydroxyproline-nitrogen ratio gradually increased, reaching a pre-treatment value by about the twenty-fourth day. The reduction in the hydroxyproline-nitrogen ratio amounted to approximately 30 per cent of the pre-injured value. There were also decreases in the hydroxyproline-nitrogen ratio of the non-injured areas and this followed the pattern of the injured skin, though much smaller in magnitude, having a maximum decrease of only about 15 per cent of the pre-treatment value.

In contrast to changes in the hydroxyproline, the ratio of hexosamine to nitrogen in the tissue increased following injury, reaching a maximum at about the third day. The ratio then decreased rapidly and reached a level approaching the pre-injury value at the time the eschar was forming. From then on, except for minor fluctuations, the values remained slightly elevated above the pre-injury level until about the twenty-fourth day following the induction of the inflammation.

The decrease in hydroxyproline by the third and fourth day post-injury was interpreted as indicating a marked loss of collagen of the skin. With this large breakdown of collagen, one might expect a release of hydroxyproline from the collagen and an increased level in the blood. Such an increase was found with one peak level occurring at the third or fourth post-injury day and a larger increase (seven times normal) occurring on the sixth or seventh post-traumatic day.

The increased hydroxyproline levels which occurred in the serum following the injury were not due to the free amino acid, but believed to be caused by hydroxyproline bound in a large molecular group such as a polypeptide. This explanation was based on the fact that the ratio of hydroxyproline to nitrogen in the serum was similar whether the determination was made on pooled serum or whether it was carried out on protein precipitates produced by adding excess amounts of ethyl alcohol.

These investigators believe that the increase in tissue hexosamine corresponds with the histological changes in which granulation tissue formation is occurring. The decrease in hydroxyproline can be correlated to the histological picture where there is a decrease in tissue collagen. However, at the time when there was little collagen in the wounded area, 70 per cent of the initial hydroxyproline remained in the tissue. This the investigators believed was associated with the reticulin component.

The change in hydroxyproline content of the tissue was interpreted to be an actual decrease of hydroxyproline, since the concentration of nitrogen ranged only from 3.3 to 3.6 millimoles per gram of fresh skin while the hydroxyproline decreased 260 to 300 micromoles per gram of skin. No reason was given why there was a decrease in the hydroxyproline content of the skin on the uninjured side.

Since neither croton oil nor any active fraction of the oil is water soluble, the in-

investigators do not think there is a systematic effect of the vesicant on the dermal collagen. Nevertheless, at least in the case of collagen formation, there appeared to be a generalized reaction to croton oil injections since there were decreased hydroxyproline levels in the uninjured skin.

These investigators correlated the histo-

logical changes with the chemical alterations noted in skin samples of animals having an experimentally induced chemical inflammation. While studies on the collagen, protein, and granulation tissues were possible, future investigations will undoubtedly cover more parameters involved in this problem, including changes in enzyme activity.

## RELATION OF WATER AND FOOD INTAKES

*Adult dogs maintained in a controlled environment consumed very constant amounts of water each day as long as they were in weight equilibrium. The water intake was directly proportional to the quantity of food and/or salt consumed.*

A relationship between the consumption of food and the intake of water has been recognized for many years. J. L. Strominger (*Nutrition Reviews* 15, 123 (1957)) showed that in rats the water intake is closely related to the amount of food consumed. In his rats, the water consumption was approximately twice the weight of the dried food consumed. This relationship held even in the rats that had been made hyperphagic by operative lesions in the hypothalamus. Subsequent to this work, H. M. Bruce and G. C. Kennedy (*Nutrition Reviews, loc. cit.*) found that the water intake was influenced by the level of protein in the diet. It was postulated that the higher water consumption of the rats fed the high protein diets was related to the water needed for renal clearance of the nitrogen compounds (*Nutrition Reviews* 13, 157 (1955)).

L. J. Cizek (*Am. J. Physiol.* 197, 342 (1959)) maintained adult dogs on measured food and water intakes for periods as long as six months. A dry commercial diet containing 26 per cent protein, 10 per cent fat, and 8.5 per cent minerals was given to the dogs in amounts required to maintain them at constant weight. The diet was mixed with an equal volume of water. The water consumed during each 24-hour period was measured, allowing for that lost by evaporation. Cizek (*loc. cit.*) points out that dogs usually drink promptly after eating, and

this represents a major portion of the water intake.

Daily water consumptions for one dog over a six-week period showed a range from 18.2 to 35.0 ml. per kg. of body weight. In spite of this marked variation in the daily consumption, weekly averages for this dog ranged from  $23.7 \pm 4.6$  to  $27.4 \pm 4.4$ . There were only slight variations in the body weight of the animal throughout this period. Consequently, the fluctuations in water consumption were due to some other factor.

The variation in water consumption over a six-week period was considerably less among other dogs. For instance, one dog showed a day-to-day range from 25.4 to 27.5, with an average of 26.3 ml. per kg. of body weight. The constancy of water intake when the food consumption remained the same was maintained in some adult animals for periods as long as four years.

In spite of the constancy of the individual water consumption, there were marked variations between individual dogs. This was shown in the average daily water consumption of 12 adult dogs (six males and six females) fed the same diet in amounts that maintained their weight. The average daily water consumption over a six-week period ranged from 22.5 to 35.7 ml. per kg. for the different dogs.

In another series of experiments, 100 g. of the dry stock diet was mixed with  $\frac{1}{4}$ ,  $\frac{1}{2}$ ,  $\frac{3}{4}$ .

1,  $1\frac{1}{2}$ , 2, 3, 4 or 5 parts of water, and each water-diet mixture was fed daily for ten days. As the water content of the diet increased, the amount of water consumed apart from that in the diet mixture decreased. The reduction was such that the total amount of water remained constant until the diet containing 2 parts of water was fed. Thereafter, however, continued addition of water to the food caused no further decrease in drinking response so that the total water consumption began to increase.

The addition of salt to a constant amount of dry commercial diet resulted in an increase in water consumption. This was proportional to the amount of salt added, which was from  $\frac{1}{2}$  to 25 g. per day. It may be assumed that the dogs were maintained on each salt level for ten days since Cizek stated (*loc. cit.*) that all "observations be done in multiples of ten before any of the material was to be used as adequate and reliable evidence." The dogs tolerated even the highest level of salt and showed "no untoward physiological effects."

Another series of experiments demonstrated that the water consumption of the dogs was always proportional to the amount of dry food consumed. There was, in fact, a straight line relationship between water consumption and the weight of food when this ranged from 0 to 500 g. per kg. of body weight, and this linear relationship was maintained even though the water content of the diet was altered. This again indicated the accuracy with which the dog adjusted the water it drank so that the total water intake remained constant for a specific weight and type of food.

It was also found that in the total absence of food, the dogs reduced their water consumption. While the water consumption remained fairly level at 25 ml. per kg. of body weight during the control week when the dogs received a constant weight of food, as soon as the food was removed the water consumption dropped to about 15 ml. for

the first day or so. It then gradually increased until toward the end of the week it was 20 ml. When food was again given to the animals after the one-week starvation period, the water consumption immediately rose to 39 ml. and then gradually decreased during the remainder of that week until it reached the pre-starvation level.

Guinea pigs and rabbits are exceptions in the preceding reaction to food deprivation. Earlier work by Cizek (*Am. J. Physiol.* 179, 104 (1954)) indicated that when all food, but not water, was removed from guinea pigs, they developed an extreme polydipsia which led to a mortality of 50 per cent of the animals by the third day of starvation and 70 per cent by the fifth day. "The animals develop severe quivering, rear up on their haunches and then topple over" with death occurring during a convulsive seizure. When guinea pigs were deprived of both food and water, there was no mortality during the five-day study.

Rabbits deprived of food, but permitted water, also developed polydipsia but showed no neurological symptoms. However, some rabbits in the course of a 13-day starvation period became edematous (H. L. Rosenthal and L. Cravitz, *J. Nutrition* 64, 281 (1958)). It is of interest that this development of edema during the withholding of food does not occur in other animals (A. Keys *et al.*, *The Biology of Human Starvation*, Vol. 2, p. 921. Univ. of Minnesota Press, Minneapolis, 1950.)

There is no explanation for the unusual behavior of guinea pigs and rabbits deprived of food. Those guinea pigs that survived the starvation period with unlimited water intake showed no apparent change in the percentage of total body water present in the gastrointestinal tract; it was 17 per cent for both males and females as compared with 17 per cent for males and 20 per cent for females receiving food and water *ad libitum* (Cizek, *Am. J. Physiol.* 179, 104 (1954)).

Cizek has suggested that the explanation for the relation between water and food consumption may lie in the osmotic pressure of the diluted food contents in the gastrointestinal tract (*Am. J. Physiol.* **197**, 342 (1959)). In addition, the requirement for renal excretion as influenced by the composition of the diet may also play a role.

All animals in the above studies were maintained under standardized laboratory conditions. Consequently, the fluctuations in water consumption that can result from

variations in activity and external temperature were eliminated.

These observations on the relationship between water intake and food consumption accentuate the hiatus in our knowledge concerning the factors that control both thirst and appetite at such constant levels. They emphasize very vividly the fact that when food consumption remains constant, the water intake is regulated with considerable constancy and precision. It will remain for future research to explain the mechanisms involved in these phenomena.

### IN VITRO ENHANCEMENT OF TISSUE UPTAKE OF VITAMIN B<sub>12</sub> BY INTRINSIC FACTOR

*Uptake of vitamin B<sub>12</sub>-Co<sup>60</sup> by rat liver slices and small intestine is enhanced by incubation with hog intrinsic factor concentrate in the presence of calcium. This permits quantitative studies and in vitro assay for intrinsic factor.*

W. B. Castle's original pernicious anemia hypothesis, that extrinsic factor interacts with intrinsic factor to form the "antipernicious anemia principle", has been modified with the finding that vitamin B<sub>12</sub> (cyanocobalamin) is both extrinsic factor and antipernicious anemia principle. Thus the function of intrinsic factor apparently is to facilitate the absorption of cyanocobalamin and possibly also to form a more potent complex. The evidence is against the latter.

The binding of vitamin B<sub>12</sub> by gastric juice has been reviewed (*Nutrition Reviews* **15**, 90, 179 (1957)). However, many other substances bind the vitamin, and assays based on this principle are therefore not valid. Intrinsic factor concentrates have been made with activity in milligram amounts; the activity is evidently quite specific, for gastric juice does not increase the absorption of folacin or carbohydrate or protein in pernicious anemia.

There are observations suggesting that intrinsic factor may prepare the intestinal mucosa for the absorption of cyanocobalamin. The relation between the two substances is not stoichiometric. Accumu-

lating evidence suggests that intrinsic factor may be a mucoprotein or mucopolypeptide. One of the difficulties in purifying and studying this material has been the lack of a simple assay for intrinsic factor. Currently used assays are based on the effect of intrinsic factor concentrates on the absorption of radioactive cyanocobalamin in man or animals (*e.g.* rats). This has supplanted the classical "reticulocyte response" method to a large degree.

However, there is accumulating evidence of species differences in several respects. Patients have been described who became refractory to hog pyloric mucosa or hog intrinsic factor concentrate, and who still responded to human gastric juice. The subject of refractoriness has been previously reviewed (*Nutrition Reviews* **16**, 235 (1958)). Another review (*Ibid.* **16**, 307 (1958)) discusses "intrinsic factor and absorption of vitamin B<sub>12</sub>" and some of the differences between the action of hog, rat and human intrinsic factor in the rat and in man.

The question of species specificity is further complicated by the presence of various binding substances, including excess

intrinsic factor itself, in almost any preparation of intrinsic factor (see below).

Since vitamin B<sub>12</sub> is effective locally when injected into the marrow, and since intrinsic factor has seemed so obviously concerned with absorption, there has been little effort to find out whether the latter has any tissue effects. O. N. Miller and F. M. Hunter (*Proc. Soc. Exp. Biol. Med.* **96**, 39 (1957)) showed that hog intrinsic factor concentrate increased the uptake of Co<sup>60</sup>-labeled cyanocobalamin by rat liver slices.

V. Herbert has pursued this line of study and published several papers on the subject. One is entitled "Studies of the Mechanism of the Effect of Hog Intrinsic Factor Concentrate on the Uptake of Vitamin B<sub>12</sub> by Rat Liver Slices" (*J. Clin. Invest.* **37**, 646 (1958)). The liver slices were incubated in Hastings' bicarbonate buffer to which hog intrinsic factor concentrate and Co<sup>60</sup>-labeled vitamin B<sub>12</sub> were added in fixed amounts. Control experiments were run with hog intrinsic factor concentrate replaced by saline. The results were expressed as retained radioactivity of washed slices (counts per minute per gram of liver slices).

Herbert found as much as ten times the uptake of Co<sup>60</sup>-labeled vitamin B<sub>12</sub> by liver slices incubated with hog intrinsic factor concentrate as by the control slices. The buffer used was not important; 0.9 per cent sodium chloride containing calcium chloride was as good as any. Enhancement of uptake by the intrinsic factor concentrate was abolished by heating the liver slices or heating the concentrate. Dinitrophenol did not diminish the enhancement effect. Uptake in the presence of the intrinsic factor increased with time for the first hour, leveling off after three hours or more. The enhancement effect was comparable at 3°C and at 37.5°C.

Replacement of various buffer ions, except for calcium, had no effect on enhancement. When this ion was replaced by an equimolar concentration of potassium, the intrinsic factor concentrate decreased uptake (40 counts per minute compared with 390 for the con-

trol slices). A number of ions, including magnesium, were not effective substitutes for calcium but strontium was. To test the effect of a chelating agent, standard one-hour incubations were performed, followed by a second one-hour incubation with added sodium chloride or ethylenediaminetetraacetate.

Ethylenediaminetetraacetate eluted no more radioactive material from the control slices than did sodium chloride. However, most of the labeled vitamin taken up by slices incubated with the concentrate could be removed by ethylenediaminetetraacetate. In other experiments, no enhancing effect was observed when binding substances such as heparin, chondroitin sulfate or orosomucoid were used in place of the intrinsic factor concentrate.

Later work by Herbert (but published earlier) is entitled "Development of a Possible *in vitro* Assay for Intrinsic Factor" (*Proc. Soc. Exp. Biol. Med.* **97**, 668 (1958)). He used rat liver slices in Krebs-Ringer solution with 0.1 molar tris-hydroxyaminomethane buffer and calcium chloride added to a standard concentration. Determinations were made at 0°C, as enhancement was more striking at this temperature; in addition, the slices did not become friable in the cold. The Co<sup>60</sup>-labeled cyanocobalamin was added in fixed amounts and the amount of the intrinsic factor concentrate varied. The two substances were added simultaneously before incubation, or in sequence (one hour's incubation followed by washing and a second hour with the second mixture). The results were again expressed as retained radioactivity of washed slices.

An optimal concentration of the intrinsic factor concentrate was found; greater or lesser concentrations resulted in less enhancement of cyanocobalamin uptake in the simultaneous incubation system. The most striking finding was that liver slices incubated for one hour with the concentrate and a second hour with labeled vitamin B<sub>12</sub> took up much more than slices incubated in the two materials together. After simulta-

neous incubation, a second hour with the vitamin increased the uptake to levels found in sequential incubation. When different preparations of the intrinsic factor concentrate were used with sequential incubation, the retained radioactivity correlated well with relative potencies as assayed by Schilling's urinary excretion test.

Gastric juice from two pernicious anemia patients had no effect on uptake of labeled cyanocobalamin; juice from a patient with nutritional vitamin B<sub>12</sub> deficiency and one with malabsorption syndrome did enhance the uptake. All four patients had histamine-fast achlorhydria. Neither heparin nor chondroitin sulfate enhanced uptake; the incubation system is therefore not a measure of binding per se.

The author recognizes that inhibitors or competitors of intrinsic factor may be present in the concentrates used; he points out that despite these possibilities his findings may well serve as an *in vitro* assay for intrinsic factor.

Herbert interprets his findings as suggesting that the hog intrinsic factor concentrate enhances uptake by physical rather than metabolic means, and that the action is not a nonspecific mucopolysaccharide or mucoprotein effect. He derives a working hypothesis, that intrinsic factor has free binding sites which attach to receptor sites on liver (in the presence of calcium), leaving other sites on the intrinsic factor molecule free to bind vitamin B<sub>12</sub> (see below).

This hypothesis is further elaborated in a later paper by Herbert entitled "Mechanism of Intrinsic Factor Action in Everted Sacs of Rat Intestine" (*J. Clin. Invest.* **38**, 102 (1959)). The same mechanism appears to operate in the small intestine and in the liver slices.

The experimental protocols were the same as those used in the previous work with liver slices. The small intestine from freshly sacrificed rats was turned inside out, washed in ice-cold 0.9 per cent saline solution and cut into segments, each tied at both ends to avoid serosal vitamin B<sub>12</sub> uptake. Incubations

were performed at 6°C.; the results were "strikingly similar" to those of the experiments with rat liver slices.

Simultaneous incubation showed no enhancement of cyanocobalamin uptake by the hog intrinsic factor over a 40-fold range of concentration. Enhancement was marked with incubation in sequence (first with the concentrate, then with vitamin B<sub>12</sub>-Co<sup>60</sup>) but there was little or none when tenfold or 100-fold higher or lower concentrations of the intrinsic factor were used.

Thus, both in the rat liver and small intestine experiments, enhancement of vitamin B<sub>12</sub>-Co<sup>60</sup> uptake was most marked with sequential incubation; it also took place in the cold, was favored by calcium and was partially reversible by later incubation with ethylenediaminetetraacetate. No enhancement was observed in calcium-free media in the case of liver, but some did occur in the case of small intestine. The author speculates that this may have been due to the difficulty of washing the intestinal mucosa free of calcium.

In other experiments on small intestine segments, the enhancing effect of the intrinsic factor concentrate on the vitamin uptake was abolished by preheating the concentrate at 100°C or by incubation at pH 1.85. 5,6-Dimethylbenzimidazole, with or without cobaltous chloride, did not affect vitamin B<sub>12</sub>-Co<sup>60</sup> binding. The author infers that these portions of the cyanocobalamin molecule may not be involved in the interaction with intrinsic factor.

The similarity of Herbert's results in the liver slice and small intestine experiments suggested to him that intrinsic factor had the same action in both loci. He explains his working hypothesis schematically, visualizing the intrinsic factor molecule as a mid-piece which, on one side, locks into receptor sites on liver or intestine in the presence of calcium. On the other side of the molecule is a (different) receptor site for cyanocobalamin, the tailpiece. "Possession of these two properties simultaneously may be unique to intrinsic factor. Heparin, chondroitin sulfate

and orosomucoid do not increase vitamin B<sub>12</sub>-Co<sup>60</sup> uptake" by either small intestine or liver slices, indicating that "enhancement of vitamin B<sub>12</sub> uptake is not a nonspecific property of any mucopolysaccharide or mucoprotein."

The author pictures the "ideal" situation in which there would be identical numbers of receptor sites, intrinsic factor molecules and vitamin B<sub>12</sub>-Co<sup>60</sup> molecules so that maximal absorption of the vitamin would take place. If too little intrinsic factor were present there would be less cyanocobalamin uptake. To explain the phenomenon of decreased vitamin B<sub>12</sub> absorption in the presence of an excess of intrinsic factor *in vivo*, Herbert visualizes some liver receptor sites occupied by intrinsic factor to which no vitamin is attached, while the incubation medium still contains cyanocobalamin attached to intrinsic factor molecules for which no mucosal site is available. Thus all intestinal receptor sites would be occupied, but not all by vitamin-binding intrinsic factor.

Incubation in sequence would bind intrinsic factor to liver or small intestine and, after washing, none would be left for the cyanocobalamin to attach to except that already bound to liver or small intestine. Not all of the facts are explained by this theory, *e.g.*, lack of enhanced vitamin uptake by small intestine after sequential incubation with large amounts of hog intrinsic factor concentrate. Herbert speculates that the intrinsic factor may be trapped between villi and later released "to compete for the vitamin with the intrinsic factor on receptor sites."

Although one could object to Herbert's

theory, he finds it helpful (when applied to intestinal absorption) in explaining a number of disputed points about species specificity. Since intrinsic factor preparations are generally contaminated with other binding materials, vitamin B<sub>12</sub>, in any simultaneous incubation experiment, distributes itself between intrinsic factor and the other binding materials. A large enough excess of otherwise potent hog intrinsic factor concentrate can bind all the receptor sites, as explained above, leaving a free excess to compete for cyanocobalamin and make it non-absorbable, whereas a lesser dosage of intrinsic factor preparation or a later dose of vitamin B<sub>12</sub> might allow absorption of the vitamin. Herbert reasons that if too much intrinsic factor can saturate the cyanocobalamin, there is considerable doubt whether species specificity is important. "Finally, the demonstration that hog intrinsic factor concentrate will enhance vitamin B<sub>12</sub> uptake by rat small intestine further weakens the . . . concept of species specificity of intrinsic factor as a phenomenon of major importance."

Herbert has performed a valuable service in investigating the action of intrinsic factor in the liver and will broaden our outlook, since most investigators have assumed that this substance acts only at the intestinal absorptive epithelium. If the liver is a physiologic site, this is useful information. If not, then comparison with similar experiments on the intestine should furnish significant differences with which to improve our understanding of the physiology of intrinsic factor. Herbert's theory will be a stimulus to further work.

### EFFECT OF CARBOHYDRATE SOURCE ON CHOLESTEROL AND CARDIOVASCULAR SYSTEM

*Rabbits fed lactose and cholesterol developed significantly more aortic atheroma, with higher serum and hepatic cholesterol levels, than did rabbits fed sucrose and cholesterol.*

Increasing numbers of nutritionists, experimental and clinical alike, have for some

time now discarded the once current but unfortunate concept of dietary carbohydrate



as only supplying caloric needs (so-called "empty calories"). Evidence is now abundantly at hand to indicate that the kind of carbohydrate may have profound influences on protein and lipid metabolism in both health and disease (M. W. Marshall and M. Womack (*J. Nutrition* **52**, 51 (1954); G. E. Peterson, E. C. Dick and K. R. Johansson, *Ibid.* **51**, 171 (1953); *Nutrition Reviews* **13**, 23, 25 (1955); **17**, 93 (1959)).

Two years ago dietary lactose was found to increase the intestinal absorption of cholesterol in the rat (W. W. Wells, *Fed. Proc.* **16**, 402 (1957); Wells and S. B. Cooper, *Arch. Biochem. Biophys.* **75**, 273 (1958)). This group (Wells and S. C. Anderson, *J. Nutrition* **68**, 541 (1959)) has now taken a logical step forward in these investigations by contrasting the atherogenic effect of lactose with that of sucrose (29.35 per cent) in diets of rabbits supplemented with 0.35 per cent cholesterol (18 animals in each group). Pathologic lesions were correlated with levels of cholesterol in the animals' sera and livers. These various food mixtures all contained cottonseed oil (13 per cent) and cod liver oil (2 per cent) as the dietary fat. Soybean meal (45 per cent) was used in place of casein as the source of protein because it had been found previously that rabbits of both groups consumed the diet best when soybean was used. All animals gained weight under these conditions, those receiving the lactose-containing diet to a somewhat lesser degree than those in the sucrose-fed group.

Despite this lesser degree of weight gain by animals in the lactose group, significantly more severe degrees of atheroma were found on gross examination of their aortas seven to eight weeks after initiation of these dietary regimens than were present in animals in the sucrose group. Measurements of surface areas of intimal lesions by three independent observers revealed that changes in the vessels of the lactose-fed rabbits were five times as extensive as those in the aortas of rabbits fed sucrose. Cholesterol contents of livers and sera in the latter were, as might be ex-

pected, significantly lower than in the rabbits on the lactose-containing diets.

The authors point out that the increased atherogenesis in the lactose group could not be ascribed to a higher absolute intake of cholesterol because this group actually consumed 2 to 4 g. less of the diets daily than did the rabbits in the sucrose group. Differences between the two groups in the amounts of cholesterol in sera and livers as well as in the relative severity of aortic atheromatous changes were far too great to be ascribed to either physiologic or chance variation.

Intestinal motility was shown, by a special technique, to be greater in the lactose-fed animals than in the sucrose controls, although diarrhea was absent in both groups. Therefore, the greater absorption of cholesterol from the intestines of the lactose-fed animals could not be attributed to lesser motility. The authors suggest that a contaminant of the lactose used (possibly lactaminic acid) might have increased cholesterol absorption, and they are repeating their study using a purified lactose. We hope it will be possible to measure cholesterol absorption more directly in these new experiments.

In suckling rabbits levels of serum cholesterol are elevated (J. H. Bragdon, *Circulation* **5**, 641 (1952)), and chickens fed sucrose-cholesterol diets develop higher cholesterol levels in both sera and livers than do chickens fed glucose-cholesterol food mixtures (D. Kritchevsky, R. R. Kolman, R. M. Guttmacher and M. Forbes, *Circulation* **20**, 964 (1959)). There are old reports of high incidences of atherosclerosis in certain primitive peoples subsisting on diets containing large amounts of milk (B. Kuczynski, *Klin. Wochschr.* **4**, 39 (1925)). Many of our own American population, while not primitive, should also be regarded as heavy milk drinkers. There is a recent report, based on autopsy data, that the incidence of coronary arterial thrombosis and myocardial infarction in patients with peptic ulcers treated with Sippy diets was twice that found in a group of selected control subjects with peptic

experimental and control animals. The ratios of active to total phosphorylase in the dystrophic and normal muscle were similar, as were the ratios in white and red muscle. Glycogen levels were higher in white muscle.

The muscle of chicks receiving the basal diet contained less dry matter than the muscles of chicks receiving vitamin E. Changes in potassium and creatine content of dystrophic muscles were variable. The authors explained decreases in these components as functions of increased water content in the muscles.

This is in contrast to the relatively large decrease in creatine observed earlier in other animals (Goettsch and E. F. Brown, *J. Biol. Chem.* **97**, 549 (1932); K. L. Blaxter and R. F. McGill, *Vet. Rev. Annot.* **1**, 91 (1955)). Creatine was significantly lower in the dystrophic muscles, as expressed on a wet basis only. The sodium content of the breast muscles of vitamin E-deficient chicks was higher in terms of both wet and dry weight. An increase in total ash in the vitamin E-deficient chicks was not statistically significant.

It is the role of phosphorylase in the synthesis and degradation of muscle glycogen that warrants a study of this enzyme in conditions of muscular dystrophy. The authors note that the activity ratios of phosphorylase were similar in normal and dystrophic muscle. Thus, the phosphorylase *a* remains proportionately constant. This situation prevails in mice with hereditary muscular dystrophy. Other studies, however, indicate that changes in phosphorylase *a*, which, of course, result in ratio alterations, can be brought about by extensive contraction of muscle.

The authors point out that, although in the case of vitamin E deficiency it might seem logical to relate the changes in phosphorylase to muscle glycogen levels, the situation does not hold for the hereditary dystrophic mice. In the latter, changes in phosphorylase parallel those found in the vitamin E-deficient chicks. Yet the concentration of glycogen in the dystrophic mouse muscles was higher than in the controls. Thus it is not possible to conclude that all muscular dystrophies are caused, directly or indirectly, by common deficiency states.

## LINOLEIC ACID AND CHOLESTEROL METABOLISM

*The addition of linoleic acid to a stock rat diet increases the incorporation of radioactive carbon in liver cholesterol and increases the excretion of the components of cholesterol catabolism.*

The ingestion of unsaturated fats causes a fall in serum cholesterol in man while saturated fats may give rise to elevation of serum cholesterol.

In order to gain information about the mechanism of the above effect, J. M. Merrill (*Circ. Res.* **7**, 709 (1959)) determined the effect of feeding saturated and unsaturated fatty acids on the incorporation of 1-C<sup>14</sup>-acetate into liver cholesterol and the effect on excretion of cholesterol metabolites.

Male Sprague-Dawley rats were fed a stock diet with 10 per cent commercial

linoleic acid or coconut oil added, or stock diet alone. After eight to 19 days, 6 microcuries per 100 g. body weight of 1-C<sup>14</sup>-sodium acetate were injected 30 minutes before death. Livers were extracted for three minutes with chloroform in a tissue shredder at 14,500 r.p.m., filtered and re-extracted. The filtrates were evaporated in an air stream and cholesterol was precipitated as digitonide. After repeated washing and centrifuging, the purified dried cholesterol digitonide was weighed and an aliquot counted in a windowless flow counter.

For comparison with the *in vivo* experiments, liver slices of rats receiving the various diets were incubated with 12.5 microcuries of sodium acetate-1-C<sup>14</sup> in Krebs-Ringer phosphate buffer at 37°C and 100 per cent oxygen atmosphere. Cholesterol was isolated and radioactivity determined.

Excretion of metabolic products of cholesterol metabolism was determined on frozen, homogenized 1 g. samples of feces taken from 24-hour collections in each of three groups of animals. After chloroform extraction, aliquots were analyzed for Lieberman-Burchard chromogens, 3-beta-hydroxy sterols and bile acids.

In the *in vivo* experiments, an increase of 259 per cent in radioactivity of liver cholesterol occurred with the addition of linoleic acid to the diet.

By sacrificing animals at 15, 30 and 45 minutes after injection of radiocarbon and analyzing the liver cholesterol, it was possible to compare the rate of incorporation of radioactive carbon in cholesterol. In rats fed a stock diet with added linoleic acid, the radioactivity reached a peak in 15 minutes and fell in the next 30 minutes. Radioactivity of cholesterol from rats fed the stock diet alone did not reach a peak until 30 minutes following injection.

Determination of radioactivity of cholesterol in animals sacrificed 120 minutes after injection showed 2.87 counts per second per mg. in control samples compared with 3.97 counts per second per mg. in linoleic-fed animals. Similarly, in animals sacrificed 240 minutes after injection the control cholesterol radioactivity was 3.0 counts per second per mg. compared with 5.5 counts per second per mg. in linoleic acid-fed animals. These results were not statistically significant.

When comparing animals receiving saturated fat with control, stock diet-fed animals, the cholesterol digitonide radioactivity of the stock animals after eight days was 1.52 counts per second per mg. compared with 1.09 counts per second per

mg. for the coconut oil group. Thus specific activity of liver cholesterol was decreased by about 30 per cent in animals receiving the saturated oil supplement.

The *in vivo* observations were confirmed by *in vitro* studies of incorporation of radioactive carbon into cholesterol. After one week on linoleic acid or coconut oil-supplemented stock diets, animals were sacrificed and liver slices incubated and analyzed. In animals ingesting linoleic acid the radioactivity was 71 per cent higher than in animals eating the diet with added coconut oil.

Average free cholesterol for stock diet animals was 205 mg. per 100 g. fresh liver weight. Animals receiving 10 per cent supplements of linoleic acid for eight days had liver free cholesterol values of 260 mg. per 100 g. fresh liver weight. Previous reports by J. Avigan and D. Steinberg (*Proc. Soc. Exp. Biol. Med.* 97, 814 (1958)) indicated that corn oil feeding increased the esterified cholesterol of rat livers but that no significant changes occurred in liver cholesterol of rats fed coconut oil.

Fecal excretion of Lieberman-Burchard chromogens increased by 47 per cent in linoleic acid-fed rats as compared to those receiving the stock ration. Similarly, the excretion of digitonin precipitable 3-beta-hydroxy sterols increased 87 per cent and bile acids increased 100 per cent. Addition of coconut oil resulted in a slight decrease in Lieberman-Burchard chromogens, but there was a 20 per cent increase in excretion of bile acids.

This type of approach may help to explain some of the paradoxes resulting from linoleic acid feeding. It would appear that the synthesis of cholesterol by the liver is considerably increased under these circumstances and that cholesterol catabolism is also greatly accelerated.

It is difficult to compare the effects of linoleic acid feeding on the anabolic and catabolic aspects of cholesterol metabolism

in this study. But it does seem that the saturated fatty acids in coconut oil produce an opposite effect on cholesterol synthesis and a similar but less marked effect on

cholesterol catabolism. Efforts to measure cholesterol synthesis and cholesterol breakdown simultaneously and in comparable units are to be encouraged.

### AN ENDOCRINE EFFECT OF NUTRITIONAL DEFICIENCIES— DEPRESSION OF HEPATIC INSULINASE

*Rats deficient in pantothenic acid or protein or riboflavin have less hepatic insulinase than normally. Deficiency of thiamine is without effect.*

Under conditions of semi-starvation or malnutrition, mature men have been observed to develop some degree of feminization. This clinical observation gains significance when one considers certain mechanisms of endocrine control. In rats, estrogenic substances are normally inactivated by the liver, usually by esterification. Whether this same mechanism applies to man is debatable. Nevertheless, when men are given a diet which is inadequate or deficient for a prolonged period of time, evidences compatible with greater estrogenic activity become manifest in loss of libido, gynecomastia and decreased growth of beard. Contrary to expectation, however, vascular spiders, often seen in conditions of estrogenic excess, were reported by W. B. Bean to be fewer per person in patients with nutritional deficiencies than in patients with chronic disease of the liver (*Vascular Spiders and Related Lesions of the Skin*, p. 83. Charles C. Thomas, Springfield, Illinois (1958)). Hepatic integrity, both nutritional and anatomic, seems to be necessary to maintain a normal rate of estrogenic destruction.

Other hormones are known to be inactivated by the liver. Hepatic destruction of testosterone is a normal function which is decreased by half in rats deprived of both niacin and tryptophan (M. J. Bryson, L. T. Samuels and H. C. Goldthorpe (*Endocrinology* **47**, 89 (1950))). Furthermore, the liver of normal animals serves as the chief inactivator of antidiuretic hormones (W. J. Eversole, J. H. Birnie and R. Gaunt,

*Ibid.* **45**, 378 (1949)). This may be one of the factors responsible for the formation of ascites by patients with Laennec's cirrhosis (E. P. Ralli *et al.*, *J. Clin. Invest.* **24**, 316 (1945)). Other studies of rats deficient in protein or certain vitamins have shown that they respond poorly to the diuretic stimulus of water loading.

A few years ago, I. A. Mirsky, G. Perissutti, and R. Jinks (*Endocrinology* **56**, 484 (1955)) demonstrated that the liver of normal animals contains a substance which will inactivate insulin. By inference, one might suspect that the apparent oversensitivity to insulin encountered in certain forms of hepatic disease could be due in part to impairment of insulinase activity. More likely however, is the fact that an injured liver cannot perform gluconeogenesis or store glycogen properly.

In a recent report, D. Diengott, S. Halevy and K. Guggenheim (*Endocrinology* **65**, 602 (1959)) described the effects of deficiencies of certain nutrients upon the hepatic insulinase of rats. Young white male rats were fed a normal diet or diets deficient in protein, thiamine, riboflavin or pantothenic acid. The group deficient in pantothenic acid was given 15 mg. of the calcium salt of omega-methylpantothenic acid per kg. of food, and the group deficient in riboflavin was given 50 mg. of galactoflavin per kg. of food. Protein was fed as casein in amounts of 0, 6, 12 or 18 per cent of the dietary weight. Insulinase activity was determined by the method described by Mirsky

(*loc. cit.*). This activity was expressed as the number of units of insulin destroyed by a gram of liver homogenate in 30 minutes at 37°C., and as units destroyed per gram of hepatic nitrogen. The authors wisely employed two groups of control animals; one group was pair-fed and the other was fed *ad libitum*.

The time it took for the experimental animals to develop evidences of deficiencies was as follows: two weeks for protein, four to five weeks for thiamine, six to eight weeks for pantothenic acid and eight to ten weeks for riboflavin. The animals were then sacrificed.

Pair-fed animals consistently had less hepatic insulinase than did the animals allowed to eat more, although the difference was slight. This also demonstrated another characteristic of hepatic insulinase. As the rats grew older and heavier their hepatic insulinase increased in concentration (both in units of insulin destroyed per gram of liver and in units destroyed per gram of hepatic nitrogen). Increasing increments of protein in the diet (from 0 to 18 per cent) resulted in significant progressive increments of hepatic insulinase.

Rats deficient in thiamine did not have impairment of hepatic insulinase function. However, those deficient in pantothenic acid or protein or riboflavin were found to have significant impairment of this enzymatic function. Riboflavin deficiency exerted the greatest effect.

The concept that hormonal actions can be potentiated by dietary deprivation is a useful one. It seems evident that several enzyme systems are involved, since a deficiency of thiamine had no effect upon insulinase but did impair inactivation of antidiuretic hormones (Guggenheim, *Metabolism* 3, 44 (1954)). Moreover, the findings of Diengott and co-workers may have significant bearing upon clinical as well as experimental problems. For example, the diabetic patient who has been labeled "brittle" be-

cause of his perverse habit of varying from a state of hypoglycemia to one of ketosis, can often benefit from an increase in his dietary protein. While most clinicians have considered that improvement resulted from a more gradual availability of carbohydrates, it is possible that more protein may provide a more constant supply of hepatic insulinase.

Furthermore, reports have appeared of abnormally low levels of blood sugar in rats deficient in riboflavin (B. R. Forker and A. F. Morgan, *J. Biol. Chem.* 209, 303 (1954)) or pantothenic acid (L. S. Hurley and J. B. Mackenzie, *J. Nutrition* 54, 403 (1954)).

In human pantothenic acid deficiency, a combination of findings appeared to indicate impairment of adrenal cortical function (W. B. Bean, R. E. Hodges, and K. Daum, *J. Clin. Invest.* 34, 1073 (1955)). However, in later studies, this group found evidence that the adrenal cortex was functionally intact, and yet an exaggerated sensitivity to insulin was consistently observed (*Ibid.* 37, 1642 (1958); 38, 1421 (1959)). The suggestion that pantothenic acid deficiency resulted in impairment of hepatic insulinase activity seems plausible.

Although deficiency of riboflavin resulted in the most marked degree of inhibition of insulinase, it took the longest period of time to develop. For this reason, a study of the several types of deficiencies for comparable periods of time would be desirable.

The biochemical actions of pantothenic acid, which functions as coenzyme A in the varied process of acetylation, and of riboflavin, which has multiple enzymatic functions, seem to have little if anything in common. Neither is it clear whether a deficiency of protein induces the same or different changes in the obscure process of manufacture of insulinase. The article by Diengott, Halevy and Guggenheim makes a significant contribution to the study of nutritional requirements and carbohydrate metabolism.

## NOTES

## Letter to the Editor

Dear Sir:

The results of our investigation (*J. Nutrition* **67**, 333 (1959)) that were cited in a recent review (*Nutrition Reviews* **17**, 340 (1959)) were not accurately interpreted. All of the evidence presented in our study would support the view that vitamin E is functioning solely as an antioxidant. Nevertheless the article was summarized in the review as "indicating the action of vitamin E is not simply as an antioxidant." A greater effectiveness of alpha-tocopherol on a molar basis when administered orally we believe is a reflection of greater retention of this antioxidant in fatty tissue compared to synthetic materials such as 1,2-dihydro-6-ethoxy 2,2,4-trimethylquinoline (ethoxyquin) and N,N' diphenyl-para-phenylenediamine (DPPD).

There has been a large number of reports in the last two years demonstrating that these particular antioxidants or methylene blue have been able to replace vitamin E in the nutrition of the chicken and the rat. When negative results are observed with antioxidants, these can be attributed to (1) using an antioxidant which does not have this property such as 2,6-ditertiarybutyl-4-methoxyphenol (BHA), 2,6-ditertiarybutyl-4-methylphenol (BHT), or propyl gallate; (2) using too low a level of antioxidant; or (3) using a level of methylene blue or N,N' diphenyl-para-phenylenediamine (DPPD) which results in toxicity and thereby prevents normal function of the animal.

There was another minor inaccuracy which we would like to correct. The reviewer consistently considered calcium DL-2-hydroxy-4-methyl thiobutyrate as an antioxidant. This material is the hydroxy analogue of methionine. Previous work (*J. Nutrition* **60**, 87 (1956)) demonstrated that appearance of muscular dystrophy of

nutritional origin in the chick is dependent on a sulfur amino acid deficiency. We do not believe, nor did we state in our paper, that this material functions other than to remedy the sulfur amino acid deficiency of the diets employed.

In our more recent work, we have found that dietary linoleic acid is a causative agent of encephalomalacia (*Poultry Sci.* **38**, 1224 (1959)). This knowledge coupled with the known role of selenium in preventing exudative diathesis has allowed us, by the following dietary treatments, to separate and produce at will any of the classical symptoms of "vitamin E" deficiency: (1) muscular dystrophy, low sulfur amino acids, low antioxidants; (2) exudative diathesis, low selenium, low antioxidants, high linolenic acid; (3) encephalomalacia, high linoleic acid, low antioxidants. In every case a suitable level of an antioxidant such as ethoxyquin will completely prevent the "vitamin E" deficiency symptoms.

In summary, we believe that all evidence to date indicates that vitamin E should be considered as a naturally-occurring biologically active antioxidant. Therefore, until data are available to the contrary, no specific or unique function can be assigned to this vitamin.

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### Effects of Excesses of Thiamine and Pyridoxine

Almost without exception, excessive doses of water soluble vitamins, even when given for prolonged periods of time, have been without detectable deleterious effect. However, M. B. Richards (*Brit. Med. J.* **1**, 433 (1945)) reported that excessive amounts of thiamine given to female rats fed a white

flour and casein diet resulted in convulsions and a high rate of mortality in their offspring. This could be prevented, however, by giving pyridoxine to the lactating mothers.

Moreover, A.D. Hunt, Jr., *et al.* (*Pediatrics* **13**, 140 (1954)) cared for an infant suffering from convulsions, whose mother had taken excessive amounts of pyridoxine during pregnancy. They relieved these convulsions by administration of pyridoxine, leading them to postulate that excessive intake of pyridoxine during pregnancy may increase the infant's requirement for pyridoxine after birth.

In a study of this problem, A. B. Morrison and H. P. Sarett (*J. Nutrition* **69**, 111 (1959)), gave excessive amounts of thiamine or pyridoxine or both (at 50 times the basal levels) to female rats. They recorded their rate of growth, reproduction performance and vitamin stores, and estimated these effects in the offspring.

The rates of growth and efficiency of food utilization in the mothers were unaffected by excess doses of the vitamins, and the average size, survival and growth of the offspring were unchanged as well.

However when both thiamine or pyridoxine were given together, the number of offspring increased slightly. There was no evidence of pyridoxine storage in the mothers, although there was considerable hepatic storage of thiamine.

When offspring of mothers who had received excess pyridoxine were fed a diet deficient in pyridoxine, they grew better than the group whose mothers had eaten a basal diet.

Determinations were made of hepatic thiamine and pyridoxine in the young rats at weaning and after two and five weeks of a pyridoxine-deficient diet. At time of weaning, the livers contained more thiamine or pyridoxine than the respective controls, although the quantity of pyridoxine was less in the young than it was in the mother.

Thus these data did not support the hypothesis of Hunt that excessive feeding of pyridoxine to mothers can increase the requirement of the offspring. However, it is conceivable that humans metabolize this vitamin in a different manner than do rats.

### Recent Books

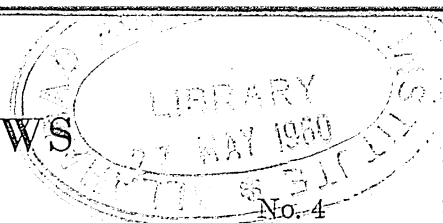
*Nutrients in Frozen Foods; Frozen Foods for Family Meals.* Prepared by Dr. Miriam E. Lowenberg and Dr. Eva D. Wilson, College of Home Economics, Pennsylvania State University, University Park, Pennsylvania. Pp. 9.

*Variation in Dental Caries Experience Among Children of Five Western States.* Cooperative Nutritional Status Studies in the Western Region III. By Gertrude Tank, Nettie C. Esselbaugh, Kathleen P. Warnick and Clara A. Storvick, Agricultural Experiment Station, Oregon State College, Corvallis, Oregon. Pp. 35.

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## SALT, FAT AND HYPERTENSION: THE JAPANESE EXPERIENCE

While pursuing studies linking salt intake to the development of essential hypertension (L. K. Dahl and R. A. Love, *A. M. A. Arch. Int. Med.* **94**, 525 (1954); *J. Am. Med. Assn.* **164**, 397 (1957); *New Engl. J. Med.* **258**, 1152, 1205 (1958)), an opportunity was made available to study the adult population of a Japanese farm village in the vicinity of Hiroshima City during February through April 1958. At this time, it was discovered that the clinical picture of hypertension was significantly different from that commonly observed in the United States. On both clinical and laboratory grounds, these Japanese had strikingly little evidence of the usual cardiac complications found so commonly in western adult populations.

Among patients with chronic arterial hypertension who die of effects related to this disease, it has been generally accepted that the chief causes of death are due to the complications of atherosclerosis in the vessels of the heart, brain and kidneys. While it is recognized that there is no necessary relationship between these two diseases, in western society atherosclerosis, particularly of the coronary arteries, is often thought to be a well-nigh inevitable concomitant of chronic hypertension. There is little doubt that in our society hypertension accelerates the progress of established atherosclerosis and it seems likely that the onset of atherosclerosis is materially hastened by the presence of hypertension.

Relative to coronary artery disease for instance, an excellent long-term, continuing study in Framingham, Massachusetts, (T. R. Dawber, F. E. Moore and G. V. Mann, *Am. J. Pub. Health* **47**, 4 (1957)) indicated that over a four-year period males aged 45 to 62 with antecedent hypertension developed about three times more clinical coronary artery disease than did males of similar age without hypertension. However, the affected

individuals appear to have been on what might be called an atherogenic regimen, i.e., a type of life in which atherosclerosis could be expected to develop in the absence of hypertension.

What is the situation when individuals with hypertension are on a nonatherogenic regimen? Most of the available experimental evidence with animals indicates that on normal diets, atherosclerosis does not develop following prolonged hypertension induced in the rat, dog, rabbit, sheep, goat and probably the chick. By contrast, when changes are effected in lipid metabolism through modifications of diet in the rabbit and chick (in the case of the dog, with the production of hypothyroidism as well), atherosclerosis develops which is clearly intensified and accelerated when the factor of hypertension is added (L. N. Katz and J. Stamler, *Experimental Atherosclerosis*, p. 224. Charles C. Thomas, 1953).

The fact that animals with chronic experimental hypertension ordinarily do not develop atherosclerosis suggests the possibility that under certain conditions, human hypertension may be dissociated from this companion disease.

I. Snapper reported that among the Chinese patients at Peking Union Medical College, atherosclerosis was uncommon and when hypertension was present, it was not associated with coronary thrombosis, although heart failure and cerebral hemorrhage were still seen (*Chinese Lessons to Western Medicine*, p. 171. Interscience, 1941). It is not clear from his brief discussion just how common either hypertension or the named complications were.

A human situation analogous to that of animals with experimental hypertension may be present in Japan where hypertension is a common and serious disease but coronary artery disease is rare, at least among the non-

professional, laboring majority of the population. In Japan, deaths from hypertension follow primarily cerebrovascular accidents as opposed to western society where deaths are largely due to cardiac complications. For instance, in B. J. Clawson's well-known large autopsy series in the United States (*Hypertension: a Symposium*, p. 239. Univ. of Minnesota, 1951), among those who were classified as dying of causes related to hypertension, 80 per cent died primarily of heart disease and only 14 per cent of cerebral hemorrhage. By contrast, in Japan at least half of the hypertensive deaths in autopsied series are due to cerebrovascular accidents (H. Ueda, *Japan Med. J.* **1740**, 27 (1957)).

Indeed, these aftermaths of hypertension have been the leading cause of death in Japan since 1951, and one of the leading causes since 1900 (S. Watanabe, *A Brief Report on Public Health Administration in Japan*, p. 13. Japanese Ministry of Health and Welfare, 1956). In 1955, Japanese males of the age group 35 to 80 had two to four times the mortality rate from this cause than American males of similar age, while the mortality from arteriosclerotic heart disease in male Japanese in this age group was from a third to a sixth as common as in their American counterparts (*Annual Epidemiological and Vital Statistics, 1955*, pp. 406-411. World Health Organization, 1958).

It is probable that differences in diagnostic standards account for some of the discrepancies in the incidence of these diseases in the two races, but it is difficult to reconcile the variations on this basis alone. For instance, in one autopsy series of about 10,000 Japanese the incidence of severe coronary atherosclerosis was approximately one tenth that of comparable individuals in the United States (N. Kimura, *Cardiovascular Epidemiology*, p. 22. Hoeber-Harper, 1956).

Furthermore, unpublished observations by American physicians of the Atomic Bomb Casualty Commission in Hiroshima are in accord with the belief that in contrast to

hypertension, coronary heart disease is relatively uncommon in the Japanese. Among the 5000 adults studied during the years 1950-55, a diagnosis of arteriosclerotic heart disease was made in 45 patients, infarction of myocardium secondary to arteriosclerotic coronary thrombosis in three, and infarction due to unknown cause in an additional eight patients. In this same series, a diagnosis of hypertensive cardiovascular disease was made in 227 patients, hypertensive vascular disease in 293, and essential vascular hypertension in 430 (Crowley, F. B., Jr., *personal communication. ABCC, Hiroshima*).

Interestingly, while the incidence of cerebrovascular accidents is generally high throughout Japan, there seem to be areas in which it is much higher than others. In general, the highest rates are in the northern areas of the main island of Honshu with progressively lower rates going southward (E. Takahaski *et al.*, *Human Biol.* **29**, 139 (1957)). This disparity in distribution is not explained by differences in climatic conditions, since the most northerly island of Hokkaido has less than half the rate of the areas noted above in Honshu. The areas in which cerebrovascular accidents are most common are also those in which hypertension is most common.

The primary cause of the cerebrovascular complications, which appear to be so common in the hypertensive Japanese, is less clear. If the lesions are primarily atherosclerotic in origin, this might suggest that the pathogenesis of coronary and cerebral atherosclerosis may be somewhat different.

If the lesions are not atherosclerotic, what are they? No clear-cut answer is available at present, but recent discussions (R. D. Adams, *Cerebral Vascular Diseases, Second Edition*, p. 23. Grune & Stratton, 1958) of hypertensive cerebrovascular disease have reemphasized the evidence for nonatherosclerotic processes such as aneurysm formation from weakened vessels. Certainly the relative mildness of atherosclerosis in the other areas of the body argues for the non-

atherosclerotic origin of some Japanese cerebrovascular accidents. It would also aid in explaining the well-known fact that, while American men have much more mortality from atherosclerotic heart disease than American women, the mortality from cerebrovascular lesions is very nearly equal in the two sexes (*Annual Epidemiological and Vital Statistics*, 1955, p. 407. WHO, 1958).

It is doubtful whether genetic factors are responsible for the striking difference in the incidence of coronary artery disease in Japanese and Westerners, although assessment of this factor is incomplete. It has been reported that the mortality rate from arteriosclerotic heart disease was higher among male Japanese living in Hawaii than among comparable men in Japan. However, Hawaiian Japanese had less disease than Japanese in continental United States, and these, in turn, had less than white Americans of similar age (T. Gordon, *U. S. Pub. Health Reports* 72, 543 (1957)). Since in this study most of the Japanese males living in Hawaii and the United States in the susceptible age group, 45 to 64, were immigrants from Japan, it is probable that such individuals remained influenced by cultural (*e.g.* dietary) patterns already established. Moreover, it is well-known that Hawaiian Japanese have retained more of their native customs than have those who migrated farther west.

A study of males of Japanese ancestry who have been exposed to a strictly western environment from birth will be a more critical test, and one might predict that arteriosclerotic heart disease will prove to be the scourge it now is among most other adult American males. Correspondence with cardiologists in Los Angeles and Honolulu suggests that this may be true at present.

There have been numerous western influences in Japan, especially since World War II, and this has included some change in diet, particularly in the large cities among the professional classes. However, the great majority of Japanese still consume the classical Japanese diet, and the current interest in

dietary factors which may play a role in the pathogenesis of atherosclerosis and hypertension warrants a passing description of this diet. From the American viewpoint, it might be most simply described as a primarily vegetarian, low fat (largely unsaturated), average protein, high carbohydrate, high salt diet (*Malnutrition and Nutrition Activities in Japan*, p. 3. FAO/WHO Nutrition Committee. Japanese Ministry of Health and Welfare. 1956). The daily fat intake of about 20 to 30 g. is derived almost exclusively from vegetables and fish and accounts for about 10 per cent of the caloric intake. Protein intakes are about 70 g. per day, of which only a quarter comes from animal sources, mostly fish and shellfish. Calories are derived mainly from cereals, of which rice is the principal source.

Salt intake is generally high and appears to decrease from north to south. In two separate studies (T. Fukuda, *J. Med. Soc. of Chiba* 29, 490 (1954); N. Sasaki, *Japanese J. Hygiene* 13, 11 (1958)), farmers in northern Honshu were found to eat about 27 g. per day, while those in middle and southern Japan averaged about 17 g. per day. (American males eat about 10 g. per day.) It is of some interest that the areas in which salt consumption is greatest appear to coincide with those areas where the prevalence of hypertension and cerebrovascular accidents is greatest (E. Takahashi *et al.*, *loc. cit.*).

If high fat and high salt intakes are important etiologic factors in the development of atherosclerosis and hypertension, respectively, it could be predicted *a priori* that the Japanese would be afflicted primarily with hypertension rather than atherosclerosis. This seems to be true.

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## NUTRITION FOR MAN IN SPACE

*The weight of nutrients needed to supply man in space becomes a tremendous problem when flights are extended for more than a few weeks or months.*

Food and drink has always been an important item for travelers, particularly in unfamiliar environments. At first the traveler's main interest is in obtaining sufficient nutrition to sustain life. After this a varied diet to eliminate monotony and supply taste preferences becomes important, and finally, if there is a plethora of nutrients, exotic foods are sought. The supplying of wholesome and appetizing foods for persons traveling on land, sea and in conventional aircraft has been solved in a relatively easy manner. However, with the advent of what the military calls high-performance aircraft, new problems of providing the travelers with desirable feeding programs are created.

Recently B. Finkelstein and A. Taylor (*Military Med.* **124**, 725 (1959)) have reported some of the problems involving nutrition for people in space vehicles. While their immediate goal is the feeding of military personnel who will be manning the aircraft of the very near future in flights of short duration, the experience gained from their studies will be used as a basis for designing feeding programs for future long-term space travel.

The type of feeding program needed for space travel will depend entirely upon the length of the flight. These authors divided the duration of flight into three categories: those of short duration (two to three days), those lasting more than three days and up to an indefinite number of months, and finally, voyages of long duration lasting for years or even generations. One of the problems of feeding under such defined flight schedules concerns the weight not only of the food itself, but of the containers and the food handling equipment and utensils.

Oxygen, not normally considered a nutritional problem, must also be supplied to the travelers. The weight of oxygen used by

man is approximately one and a half pounds per day (depending on activity), or approximately 550 pounds per year. Since man needs approximately 2.2 liters of water per day, one would have to carry almost a ton of water to supply one man for a year. Moreover, it has been calculated that an individual at home consumes approximately seven pounds of food per day, which includes the weight of the packaging, inedible portions and food lost in preparation.

Finkelstein and Taylor report how some of these problems have been solved, at least in part. For flights of only two or three days, bottled oxygen, stored water, ready-to-eat types of food (box-lunch style) prove adequate. For longer journeys, the weight problem may still be solved by present technology if the length of flight is not too long. This requires devices which break down carbon dioxide to oxygen so that it can be re-used. Water can be recovered from both the air and excreta and purified. Dehydrated foods packaged in very light containers from which the food can be eaten and equipment limited to a means of heating the package will reduce the weight of the food load drastically.

However, for flights of long duration a ration of dehydrated "quick serve" foods supplying about 2500 calories per day with the usual ratio of fat, carbohydrate and protein and adequate amounts of minerals and vitamins would weigh about 3.5 pounds. To supply one man with this type of food for one year would add about 1300 pounds of weight. Thus, for trips of longer than two to six months it would not be economical to carry foods.

Methods must be developed for regenerating carbon and nitrogen compounds and re-converting the metabolic wastes into food. A process of growing algae can be used for such a cycle, which will also produce oxygen.

In order to operate such a scheme in a space vehicle, however, the natural process must be sped up greatly and confined to a small space. While the basic research in this field already has been done, considerable increase in technology or applied research must be undertaken before such a process becomes feasible.

As a prelude to this long-term space travel, Finkelstein and Taylor have studied the problem of feeding personnel who would be manning high-performance aircraft. They studied two different groups of five men who were maintained in the confined limits of a crew compartment for five days, and also 35 individuals who were maintained in a dark sound-proof chamber from six to 168 hours. These studies simulated some of the conditions of space travel to which crews would be subjected. A study was also carried out on a subject during a high-altitude balloon flight of about 100,000 feet. In all these investigations it was possible to provide adequate nutrition under rather stringent environmental conditions.

In contrast to the normal feeding of military personnel, however, consideration must be taken of the psychological effect of food. It has been found that under stressful situations food may serve as a means of relieving stress.

Under the conditions of true space travel man will be subjected to periods of weightlessness, a condition which, according to these investigators, cannot be reproduced experimentally for more than about 45 seconds. Thus at present one cannot study

the effects of lack of gravity on the mechanics of eating, ease of digestion, absorption or even gross nutritional requirements. However, it is possible to surmise some of the difficulties and plan accordingly.

During early ventures in space, man will probably wear the full pressure suit, which includes helmet and gloves. Thus in order to feed him, it will be necessary to employ fluid diets contained in squeeze-type bottles which can be connected to a tube leading to the mouth. The particle size of the formula must be small to provide free flow and also to promote better digestion. When normal gravitational effects are removed for short periods there have been reports of disorientation, nausea and motion sickness. With the use of liquid foods under gravity-free conditions, measures must be taken to prevent the aspiration of the diet into the lungs. Also, the diet must be low in residue since pressure suits make no provisions for defecation. The group has already reported two different fluid menus which supply 2300 calories per day with a protein intake of 100 g. Test subjects on such a regimen carried out their normal duties and had insignificant weight changes during the period studied.

These investigators conclude that, while the problem of feeding personnel in high-performance aircraft and in space travel has not yet been solved, greater variety of concentrated foods, further advances in cold sterilization of foods, improved methods of packaging and the development of lightweight food service equipment will provide some solutions.

## SYNDROMES OF MAGNESIUM DEPLETION AND RETENTION IN MAN, PART II

*A syndrome of magnesium deficiency can probably be defined in certain patients with alcoholism or electrolyte imbalance. This consists of sudden psychoses, neuromuscular hyperirritability and electrocardiographic changes.*

As pointed out in part one of this review, recognition of clinical magnesium deficiency has been hampered by the poor correlation

between serum and intracellular concentrations of this ion. Investigators and clinicians can at best make inferences until some more

direct measure of intracellular levels is worked out.

Precisely this has been attempted by W. O. Smith and J. F. Hammarsten in an article entitled "Intracellular Magnesium in Delirium Tremens and Uremia" (*Am. J. Med. Sci.* **237**, 413 (1959)), in which they have shown very nicely changes in cell magnesium content in these two syndromes. The authors refer to a number of papers implicating an increase or decrease of magnesium in certain clinical situations, principally delirium tremens and uremia. They point out that the poor correlation between serum magnesium levels and these syndromes is analogous to the inconsistent results reported in the earlier papers on potassium. Smith and Hammarsten selected red blood cells as the most easily obtainable cells for their determinations, recognizing, however, that erythrocytes might not be typical of other cells of the body.

In 13 healthy adults the mean erythrocyte magnesium concentration was  $5.29 \pm 0.34$  mEq per liter; the corresponding plasma level was  $1.80 \pm 0.13$ .

Twelve patients with delirium tremens were studied. All were disoriented and confused; 11 had a "pronounced" tremor and seven were suffering hallucinations. The mean red cell magnesium concentration was  $3.9 \pm 0.75$  mEq per liter, and all values were below the "normal" range defined on the authors' charts. The mean plasma concentration was  $1.50 \pm 0.28$  mEq per liter, a significant decrease ( $p = < 0.001$ ), although five or six of the values were within the "normal" range of 1.54 to 2.06. In the nine patients treated with intramuscular magnesium sulfate there was "marked" clinical improvement within 12 to 24 hours. Seventy-two hours after this treatment, the mean red cell magnesium concentration had risen to  $5.19 \pm 1.47$  mEq per liter and the mean plasma level to  $2.20 \pm 0.40$ . The individual values are not given in the paper.

Determinations were made on 14 patients who had uremia and central nervous system

depression. The mean erythrocyte magnesium concentration was  $8.84 \pm 1.71$  mEq per liter, and all 13 values charted were above the upper limit of normal (6.00). The mean plasma level was  $3.17 \pm 1.30$  mEq per liter; ten of the 14 values were above the upper limit of "normal" on the chart and the remaining four were within the normal range.

Electrocardiograms were made on three patients with delirium tremens and nine of the uremic patients. Changes possibly attributable to abnormal intracellular magnesium concentrations were found in two thirds of these patients.

While the authors are cautious about generalizing, they conclude that at least one cell, the erythrocyte, is depleted of magnesium in delirium tremens and that "the close correlation between the hyperexcitability of the central nervous system with the lowered erythrocyte magnesium levels suggests that the brain cells may also be deficient in magnesium."

What the correlation between magnesium levels in erythrocytes and other cells may be is not discussed. The concentration in muscle cells is several times that in erythrocytes, but one does not know what obtains in various other specific tissue cells, nor whether these values change independently or in parallel fashion.

Smith and Hammarsten point out that magnesium therapy of delirium tremens requires 12 to 24 hours before clinical improvement occurs and they speculate that the delay may be due to slow cellular repletion. They point out that serum or plasma magnesium concentrations are more reliable evidence of a total body excess than of a deficiency of magnesium, as would be expected from the predominantly intracellular position of this cation.

The authors' results, although derived from a small series of cases, seem quite significant. First, needed data have been provided to those interested in electrolyte physiology. Second, the results establish



what previously had only been surmised from a confusing assortment of clinical observations and plasma levels. Lastly, they are quite consistent with what we know of the physiologic actions of magnesium. The parallel with the development of our knowledge of syndromes of potassium excess and deficiency may well be a close one.

Smith and Hammarsten are careful not to claim that the changes they have found are the cause of delirium tremens or the uremic syndrome, but it seems reasonable to believe that they are important factors. Further research will establish whether this is so.

Further work on magnesium depletion in man is reported by R. E. Randall, Jr., E. C. Rossmeisl and K. H. Bleifer (*Ann. Int. Med.* 50, 257 (1959)). They report observations on 12 patients with sudden psychiatric and neuromuscular symptoms and relate the symptoms to depletion of total body magnesium; "in ten instances the serum magnesium concentrations were notably depressed." Several patients showed evidence of depletion of body magnesium, and calcium metabolism was regularly altered. The authors cite normal serum magnesium values as 1.7 to 2.7 mEq per liter (mean 2.2). Postmortem examinations were performed in six cases and surgical biopsies in two others.

*Case 2* was a 68-year-old man with carcinomatosis, severe malabsorption and cachexia. An abdominoperineal resection was performed for carcinoma of the rectum in 1950. Heavy irradiation for metastases in 1955 was followed by diarrhea and, in 1957, cachexia. Laparotomy and biopsy showed carcinoma and "extensive post-irradiation changes"; the entire small bowel was scarred and adherent. After a period at home with continuing diarrhea and weight loss, the patient was readmitted because of weakness and "episodes of bizarre behavior." On the following day he "suddenly became irrational, disoriented, confused, and progressively noisy, combative and wildly restless," with hallucinations. Intravenous fluids were

given, with vitamins, dextrose, saline and potassium. After six days of severe psychosis "a severe neuromuscular disorder developed, characterized by muscular rigidity and cogwheeling, coarse tremor, opisthotonos, extensive fine muscle fasciculations and twitching." Very low serum levels of calcium and magnesium were discovered. Intravenous calcium gluconate caused no improvement.

Nine days after onset of the psychosis, intravenous magnesium sulfate was added to the regimen (150 mEq plus that in the diet). Eighteen hours after the first dose of magnesium sulfate "the patient was remarkably rational, oriented, and completely free of the previous neuromuscular disorder. By the third day of therapy he was up and about, completely transformed from his 'terminal' state into a delightful elderly man." Over the next five weeks, with diarrhea and no supplement of magnesium, the serum calcium and magnesium levels fell progressively again to very low levels but with no psychiatric or neuromuscular symptoms. Changes in the electroencephalogram and electrocardiogram improved slowly after treatment, but could not be correlated with electrolyte levels or clinical symptoms.

*Case 4* was a 63-year-old alcoholic man with cirrhosis. Part of the colon was removed (because of multiple polyps); there followed much gastric and fecal fluid loss, confusion on the second postoperative day and severe psychosis and twitching by the fourth. Treatment with magnesium was followed by improvement within 15 hours. Abnormalities in the electromyogram, electroencephalogram and electrocardiogram disappeared and much of the magnesium was retained.

*Case 5* was a 38-year-old diabetic male with mild chronic pyelonephritis, admitted to the hospital because of a stroke and aphasia. There had been no gastrointestinal or dietary abnormality. The patient developed severe epilepsy ("status epilepticus") on the third day, lasting for three



weeks and uncontrolled by large doses of anticonvulsant drugs. When extreme serum electrolyte changes, including very low magnesium values, were found, large amounts of several electrolytes, including calcium, potassium and magnesium, were infused. "During the first hour of electrolyte 'replacement' the status epilepticus stopped and only focal seizures occurred." Although magnesium was retained during the first 40 hours, there followed three days of large renal losses. However, there was a large retention of magnesium several times during the second and third months. The patient improved rapidly and within three weeks could walk, with residual right hemiparesis and speech defect. Electrocardiographic and electroencephalographic findings improved progressively.

Three months after admission to the hospital a myocardial infarct supervened, leading to death 11 days later. Postmortem examination showed the infarct of the brain and heart and acute and chronic pyelonephritis; the intestine was normal. Although the authors are unable to explain the marked changes in calcium metabolism, they presume that the potassium depletion was a renal loss superimposed on a deficient intake. In some way, they infer, this may have brought about simultaneous renal loss of magnesium.

Randall and his co-workers, summarizing their cases, found that all patients were "severely malnourished" for such reasons as anorexia, inability to eat, alcoholism, diarrhea and vomiting. Hypokalemia was a feature of three cases and two had hyponatremia. Eleven of 12 patients were being fed parenterally when their symptoms developed or became worse. Seven patients had sudden and severe psychoses ("delirium, hallucinations, delusions and wild, combative behavior"); four others had "mild confusion and disorientation." Withdrawal from alcohol immediately before hospital admission may have occurred in several of the alcoholic patients. Muscle twitchings occurred in all but one patient;

rhythmic tremors occurred in six, seizures in two, and only one (case 5) had physical signs of neuromuscular irritability. Ten patients had low serum magnesium levels; two had normal levels with balance data indicating magnesium depletion. Hypocalcemia was found in all 12 patients.

Psychotic and neuromuscular symptoms stopped in six cases within six to 18 hours after treatment was started; improvement was "dramatic" in three of these (including cases 2 and 4, summarized above). In several cases the symptoms and serum magnesium levels could not be correlated. All six patients tested had "diffusely abnormal" electroencephalographic tracings. Many showed depressed ST segments and low T wave voltage in the electrocardiograms. Six autopsies and two biopsies yielded no specific lesion attributable to magnesium deficiency.

Magnesium tetany (in a child) has been reviewed in this journal (*Nutrition Reviews* 2, 189 (1944)). Recently, D. D. Ulmer, W. E. C. Wacker and B. L. Vallee have reported four patients (*J. Clin. Invest.* 38, 1049 (1959) (abstract)). "True magnesium tetany" occurred in each case "as a complication of either severe infection or surgery in malnourished patients, treated with magnesium-free parenteral fluids for prolonged periods. In each, the serum magnesium concentration was low and that of calcium was normal. Parenteral magnesium resulted in prompt and dramatic amelioration of all the manifestations of the *magnesium tetany syndrome*."

The authors found the syndrome "virtually identical" to that observed in animals: "semicoma, severe neuromuscular hyperirritability including Chvostek's sign and carpo-pedal spasm, athetoid movements, marked susceptibility to auditory, mechanical and visual stimuli, a decreased magnesium and a normal serum calcium concentration." The syndrome "can only be distinguished from hypocalcemic tetany by chemical means." The importance of the observations of Ulmer's group lies in the

apparently more discrete clinical identification of the background for the magnesium deficiency. Their full report should prove illuminating.

The various authors cited in this review have reported an impressive amount of clinical research. The studies are perforce incomplete and leave many questions unanswered.

Simplified methods of magnesium determination (flame photometer, spectrography and chelation) are being developed and should before long be widely used to answer these questions. Should flame photometry, for example, prove to be a really simple and reliable method of measuring magnesium in biological fluids, one may expect many new observations among patients with electrolyte disturbances, primary aldosteronism, acute renal failure, hyperparathyroidism and others, as well as in the whole gamut of conditions associated with altered neuromuscular irritability.

The difficulties caused by the intracellular location, large body stores and renal conservation of magnesium will have to be overcome. Randall and co-workers point out that estimation of total body magnesium (*e.g.*, by the radioisotope dilution method) would be a far better measure of depletion than are balance data. The lack of correlation between serum magnesium concentration, total body stores and intracellular levels has been emphasized by all authors. No doubt erythrocyte content as measured by Smith and Hammarsten is a better indicator, but we do not yet know how satisfactory it will prove.

The known pharmacologic relations be-

tween magnesium and potassium and calcium metabolism have never been well explained; perhaps they will be soon. There is evidence that calcium or potassium administration aggravates magnesium deficiency in animals. Randall's group points out that decreases in serum magnesium levels have been observed in man after calcium administration and after glucose. Perhaps the latter phenomenon is a result of increasing intracellular glycogen, entraining intracellular ion with it. The authors point out that some of their patients had been receiving glucose, potassium or calcium in their parenteral fluids. Much remains to be elucidated before the relation of magnesium to calcium metabolism is worked out, and our speculations about bone deposition, intracellular content, phosphate, vitamin D and parathyroids are very imperfect at present.

The papers reviewed have helped considerably to define the syndrome of magnesium deficiency in man, which should soon qualify for inclusion in our textbooks. If patients with such a syndrome can be distinguished, our knowledge can be greatly advanced by the mere selection of subjects appropriate for study.

It seems fair to conclude that onset of psychosis or semicoma plus signs of tetany, especially in circumstances with starvation and fluid and electrolyte loss, should suggest the possibility of magnesium deficiency. In short, if the stage is set, onset of a delirium tremens-like syndrome justifies a chemical and metabolic search for magnesium deficiency as a prelude to specific replacement therapy.

## IRON FORTIFICATION OF MILK

*The fortification of a prepared milk formula with iron (12 mg. per reconstituted quart) has been shown to maintain hemoglobin and serum iron concentrations at normal levels in both full-term and premature infants.*

Many recent studies have emphasized the prevalence of iron deficiency in infants

between six and 18 months of age in the United States--(*Nutrition Reviews* 17, 11

(1959)). Since iron deficiency in infancy is a preventable disease, this large public health problem can be solved by the addition of adequate amounts of iron to the infant dietary. Studies by the Child Research Council in Denver (*Ibid.* 16, 67 (1958)) have demonstrated that infants ingesting large amounts of infant cereal fortified with iron have intakes generally considered to be adequate for the prevention of iron deficiency anemia.

W. L. Niccum, R. L. Jackson and G. Stearns (*Am. J. Dis. Child.* 86, 553 (1953)) demonstrated that the addition of inorganic iron to milk in amounts of 5 to 10 mg. daily would also prevent iron deficiency anemia. However, studies on the absorption of iron from milk conflict with studies in which iron is administered separately.

The following study demonstrates quite clearly that iron added to milk is absorbed in amounts adequate to prevent the development of iron deficiency anemia and to maintain serum iron concentrations that must be considered normal for infants.

A. Marsh, H. Long and E. Stierwalt (*Pediatrics* 24, 404 (1959)) studied the performance of infants during their first nine months of life on three feeding regimes. Group one was fed a prepared milk formula containing 12 mg. of iron per quart. The second group was fed the same prepared formula without added iron. A third group was fed a formula made from evaporated milk, water and sucrose at the same caloric concentration and containing no added iron. Except for small amounts of cereal of extremely low iron content, no solid foods were received by these infants during the nine months of the study. Seventy-four full-term and 42 premature infants were observed for the full nine-month period.

The hemoglobin values for the full-term infants were consistently higher in those fed the iron-containing formula after the age of three and one half months, averaging 12.69 g. per cent for the iron-supplemented group and 10.46 and 9.67 g. per cent for the

unsupplemented groups. These differences were highly significant on statistical analysis. Among the premature infants, the iron-supplemented group had hemoglobin concentrations practically identical with those of the full-term infants but the hemoglobin concentrations of the unsupplemented groups were even lower, averaging 9.4 and 8.5 g. per cent respectively for groups two and three.

Serum iron concentrations were also measured. In the full-term infants, statistically significant differences between the iron-supplemented group and the two control groups appeared after the age of five months, and as early as the age of three months in the premature infants. Statistically significant differences in the hematocrit occurred only at the age of nine months and were not as pronounced as the differences in hemoglobin or serum iron concentrations.

The iron-fortified milk preparation used was well tolerated by all the infants in the study. No objective evidence of a difference in incidence of infection or other criteria of performance was found. Mean serum iron concentrations in the supplemented group averaged about 60 micrograms per cent for both full-term and premature infants. In the unsupplemented group, serum iron concentrations fell to approximately 35 micrograms per cent in the full-term infants and to as low as 20 micrograms per cent in the premature infants. These low serum iron concentrations were not always accompanied by the development of anemia (defined for the purposes of this study as a hemoglobin concentration less than 8.0 g. per cent).

These studies were carried out under somewhat artificial conditions since supplementation of the milk diet for the first nine months of life was not allowed in the study group. The demonstration that iron added to a prepared formula at a concentration of 12 mg. per reconstituted quart will maintain normal hemoglobin concentrations in infants has confirmed previous studies,

supplementing them with data concerning the concentration of serum iron.

Although there is no doubt that iron deficiency can be prevented by the addition of iron to the milk formula of both premature and full-term infants, other foods

can also be supplemented. Certainly a great deal more experience concerning supplementation of infant dietaries needs to be accumulated before the wholesale fortification of the milk supply available to small infants can be seriously considered.

## MINERAL ELEMENTS OF FRESH VEGETABLES

*The average amounts of ten mineral elements in fresh vegetables vary widely between vegetables from different geographic areas and even from shipment to shipment from the same farm.*

Ever since the recognition of deficiency diseases, there has been concern regarding the composition of foods from different areas. The effects of native fertility, soil amendments and other environmental factors upon the composition of vegetables and fruits have been scrutinized as possible indicators of food quality.

With Washington, D. C. as a marketing center, an extensive study has been made of the mineral composition of nine vegetables from producing centers located in California, Arizona, Texas, New Mexico, Maryland, New Jersey, New York, Michigan, Delaware, Florida, North Carolina and Pennsylvania (H. Hopkins and J. Eisen, *J. Agr. Food Chem.* 7, 633 (1959)). Three or more lots of each vegetable were collected equally from major producing areas representing 90 per cent of the wholesale market during 1956 and 1957, with the exception of sweet corn and tomatoes. The seasonal delivery pattern limited collection of samples to five different vegetables at any one time, and efforts were made to secure samples shipped by different producers in a given area when samples were secured in successive weeks. Information on production and handling was secured from the label and shipping tags and from the shippers and growers.

A crate, bushel or carton of each vegetable was secured and reduced to the edible portion for analysis. The vegetables analyzed included asparagus, snap beans, cabbage,

carrots, pascal celery, sweet corn, iceberg lettuce, onions and tomatoes. These make up the bulk of vegetables, other than potatoes, used in United States households (*U. S. Dept. Agr., A. M. S. Household Food Consumption Survey Report No. 1 (1955)*). The elements determined included boron, phosphorus, magnesium, manganese, iron, aluminum, calcium, copper, sodium and potassium.

In presenting their report, Hopkins and Eisen emphasize that there may be large variations in the amount of an element found in different vegetables, or in the same vegetable from different areas, or in the same vegetable from the same area at different seasons, or indeed in the same vegetable from the same farm and the same season but from different shipments. Earlier studies have also noted the differences which occur for certain elements between lots, and G. T. Sims and G. M. Volk pointed out that these differences limited the significance of regional differences (*Fla. Agr. Expt. Station Bull.* 438 (1947)).

The rapid changes which have occurred in cultural practices within the last decade give special pertinence to the values presented by Hopkins and Eisen. The wide diversity of production areas, the selection of vegetables and the ten mineral elements for which values are reported, lend additional interest to their report. Also, most of the values for a specific element in a given vegetable had a

coefficient of variation of less than 10 per cent, suggesting a good sampling procedure.

The levels of sodium found in different areas were quite striking. Carrots and celery were particularly noteworthy, in that they had much higher values on the average than the other seven vegetables. Carrots averaged 53.0 mg. per 100 g. with a low value of 1 mg. and a high of 132 mg. per 100 g. Celery ranged from 27 to 133 mg. per 100 g. Unfortunately for those who might like to select these two vegetables for sodium content, some of both high and low values came from the same areas. However, the sodium content of the other vegetables was of an entirely different magnitude. For example, the average value of sweet corn was 0.19, tomatoes 2.1, asparagus 1.8 and iceberg lettuce 6.3 mg. per 100 g. of edible portion.

There were some large differences in area mean values for some of the elements in certain vegetables. The mean copper content of carrots from northwest New Mexico (0.05) may be compared with that of South California (0.18); the sodium of central California iceberg lettuce (13.0) with that of central New York (0.9); the manganese in tomatoes from central Florida (0.09) with that in tomatoes of Eastern Pennsylvania (0.21), all in terms of mg. per 100 g. of edible portion. However, the wide variations which may occur even with successive de-

liveries from the same farm emphasize the hazard of attributing any special significance to regional differences.

Hopkins and Eisen gave special attention to the variations in phosphorus and manganese content of fresh carrots as received in Washington, D. C. during the period April-October 1957. The phosphorus values ranged only slightly about the weighted mean of 34 mg. per 100 g. fresh weight during this period, and this was characteristic of most of the other elements. The manganese values, however, varied quite widely around the weighted mean of 0.29 mg. per 100 g., indicating a highly variable seasonal pattern for this element. A similar exceptional pattern is revealed for sodium in lettuce.

The data presented by Hopkins and Eisen partially fill a gap that has long been evident. The selection of material on an edible portion basis and from quite diverse production areas further enhances the values obtained. It is also clear that the variations in mineral element content, while not great for some elements, are tremendous for others.

Results such as these emphasize how little quantitative data are available concerning the mineral element composition of our foodstuffs, and how much less is known of the factors influencing the amounts of elements present in vegetables which come to market.

### ANTAGONISM BETWEEN ESTRONE AND PARATHYROID EXTRACT IN CALCIUM METABOLISM OF BONE

*A technique employing radioactive calcium permits detailed studies in animals of the rate of deposition in bone and of the size of the exchangeable pool. Parathormone and estrogen have opposite effects on calcium metabolism.*

Many methods have been employed to estimate the status of skeletal metabolism of calcium. In far advanced rickets or osteomalacia, the clinical appearance is sufficient evidence to establish the nature of the defect. But in mild forms of skeletal disorders, sensitive tests must be employed. Unfor-

tunately no single test or combination of these satisfies the requirement. A helpful method is provided by comparison of x-ray films of skeletal structures with films of a standard series of ivory (or metal) wedges of increasing thickness (*Nutrition Reviews* 10, 119 (1952)). However, even these tech-

niques fail to correlate perfectly with studies of calcium balance. Moreover, even the balance technique can be subject to misinterpretation, since the size of the exchangeable calcium pool will vary from one individual to another, and the rate of calcium absorption from the gut may vary from day to day.

Clearly, a more precise method was needed. Some of this gap was filled by a technique devised by G. C. H. Bauer, A. Carlsson and B. Lindquist (*Kungl. Fysiograph. Sällsk. Lund. Förh.* **25**, 1 (1955)). This method, modified slightly by R. E. Ranney (*Endocrinology* **64**, 783 (1959)), consisted of feeding a standard diet to adult male mice, administering a drug to be tested for an appropriate period of time, and injecting the animals with radioactive calcium ( $\text{Ca}^{45}$  lactate) at intervals of 144, 96, 48, 24 and four hours before sacrifice. Samples of serum and of bone (left tibia) were taken for radioactive measurement. The technique allowed estimates to be made of both the exchangeable pool of calcium and of calcium which had been deposited recently in bone (calcium accretion).

While endocrine control of calcium metabolism is certain, the details are in controversy. Studies of the pharmacologic effects of parathyroid hormone have shown that it increases the concentration of calcium in the blood and decreases that of phosphorus, while at the same time both substances are excreted more rapidly in the urine (F. Albright and E. C. Reifstein, Jr., *Parathyroid Glands and Metabolic Bone Disease*. Williams and Wilkins Co., Baltimore (1948)). These effects may be accounted for by an increased rate of renal excretion of phosphorus. This leads to increased resorption of calcium phosphate from bone, and the consequent rise in levels of calcium in the blood leads to more rapid urinary loss of calcium. Unless this loss is compensated by greater dietary intake, the result will be decalcification of bone.

In opposition to this theory, W. F. Neuman and M. W. Neuman (*Am. J. Med.* **22**,

123 (1957); *Nutrition Reviews* **15**, 188 (1957)) proposed a different explanation. Their view was that bony matrix is constantly being calcified as a result of exposure to fluids which are supersaturated with calcium salts. Balance is maintained by citric acid, formed by osteocytes in the bone, which chelates calcium and returns a portion of it to the blood. The action of parathormone would be to regulate the rate of formation of citric acid by these cells of the bone.

The role of estrogens in calcium metabolism has been equally controversial. While few deny that estrogens exert some effect, the mechanism has remained in dispute. Postmenopausal osteoporosis develops in some women deprived of their ovaries either by surgery or by senescence. Administration of estrogens (usually with androgens) may effect a cure. However demineralization of bone may occur during pregnancy, although this is presumably caused by inadequate diet.

In 1957, G. Manunta, J. Saroff and C. W. Turner (*Proc. Soc. Exp. Biol. Med.* **94**, 785 (1957); *Nutrition Reviews* **15**, 343 (1957)) reported studies performed with the  $\text{Ca}^{45}$  technique of Bauer. Administration of estradiol resulted in more radioactive calcium in the serum but not in bone, while parathyroidectomy reduced  $\text{Ca}^{45}$  in bone but not in serum. When estradiol was given to parathyroidectomized animals it increased the  $\text{Ca}^{45}$  content of serum and prevented the decrease in bone. Parathyroid extract alone decreased the  $\text{Ca}^{45}$  content of bone, but estradiol and parathyroid extract together increased the  $\text{Ca}^{45}$  content of bone. These findings were interpreted as evidence that parathormone and estrogens have an opposite effect on bone metabolism and that estrogens might alter the transport of calcium.

Electrophoretic studies of serum from these animals showed that  $\text{Ca}^{45}$  migrated with albumin and the alpha globulins in control animals, but with beta and gamma globulins in animals that had received estrogens. Unfortunately some doubt was cast upon the validity of this latter finding be-



cause of the tendency of calcium to dissociate from its carrier proteins during electrophoresis.

In an attempt to clarify the effects of estrogens and parathormone upon calcium metabolism, Ranney (*Endocrinology* 65, 594 (1959)) employed the same  $\text{Ca}^{45}$  technique. Dividing 15 groups of ten adult male mice into three groups of 50 each, he administered 1.2 mg. of estrone per kg. of body weight to group one, 165 USP units of parathyroid extract per kg. of body weight to group two, and solvent oil to group three for a total of 23 days. Then he gave subcutaneous injections of  $\text{Ca}^{45}$  lactate and obtained samples of serum and of bone. By calculation, he estimated the rate of bone accretion of calcium, the total exchangeable fraction (pool) of calcium, and the rate of resorption of calcium from bone.

His results confirmed the report of Manunta, Saroff and Turner that estrogens and parathormone had opposite effects upon the rate of accretion of calcium. However, the differences in the exchangeable fractions of calcium among the three groups were negligible. Administration of both hormones simultaneously resulted in a canceling effect. However, if the dose of parathyroid hormone was kept constant and that of estrone increased progressively, the rate of accretion rose, although less rapidly than in animals given graded doses of estrone alone.

In order to study the size of the exchangeable mineral pool, Ranney gave  $\text{Na}^{22}$  to a

group of animals treated similarly. Estrone did not affect the concentration of  $\text{Na}^{22}$  in serum but did result in greater amounts in bone. This was considered as indicating an expansion of the exchangeable calcium pool.

The author concluded that his results indicated little or no effect of parathyroid extract upon the exchangeable calcium pool. He suggested that the antagonistic action between estrone and parathyroid extract respecting the rate of bone accretion of calcium was caused by (1) an increase in the size of the exchangeable (calcium) pool by estrone, (2) the relative insensitivity of animals to small quantities of estrone but greater sensitivity to larger doses, and (3) the "action of estrogens upon bone (which) may in some way promote the inclusion of calcium citrate complexes into the stable phase of bone crystal."

This study confirms the fact that parathyroid hormone does produce demineralization of bone and that estrogens have an opposite effect. However, the assumption that  $\text{Na}^{22}$  and  $\text{Ca}^{45}$  occupy the same exchangeable bone fraction seems presumptuous. Also there would seem to be little reason to invoke theories of variable sensitivity to estrone or of alterations in citrate metabolism unless these functions were measured. While this article adds slight information to the problem of calcium metabolism, the technique of isotopic metabolic study should aid further investigators.

### VITAMIN $\text{B}_{12}$ , METHIONINE AND FAT

*Vitamin  $\text{B}_{12}$  and methionine are both shown to increase utilization of fat in the diet. The nature of their interrelationships and their roles in the utilization of fat remain obscure.*

It has been shown that raising the fat level of corn-soybean meal diet from 3 to 22 per cent will increase the severity of vitamin  $\text{B}_{12}$  deficiency in chicks (M. R. Spivey *et al.*, *Proc. Soc. Exp. Biol. Med.* 85, 451 (1954)), at the same time elevating the vitamin  $\text{B}_{12}$

requirement (M. R. Spivey Fox *et al.*, *Ibid.* 93, 501 (1956)). Fox *et al.* (*J. Nutrition* 62, 539 (1957)) have further shown that the high vitamin  $\text{B}_{12}$  requirement could be eliminated by supplementation of the diet with methionine.



However, the usefulness of the above crude diet for vitamin B<sub>12</sub> studies was limited because it did not permit the omission or variation in level of most nutrients. To overcome this difficulty, Spivey Fox, L. O. Ortiz and G. M. Briggs (*J. Nutrition* **68**, 371 (1959)) have studied the vitamin B<sub>12</sub>-sparing effect of methionine in chicks fed a purified diet containing 0, 4 and 24 per cent hydrogenated vegetable oil.

All diets used in these experiments had the following basal composition in grams per kilogram of diet: soybean protein 300, L-cystine 3, corn oil 40, salts A 60, vitamins 1, and crude glucose 596. The following vitamins were added in milligrams per kilogram of diet: thiamine hydrochloride 8, riboflavin 8, calcium pantothenate 20, choline chloride 1000, nicotinic acid 100, pyridoxine hydrochloride 8, D-biotin 0.3, pteroylglutamic acid 3, vitamin A acetate 6, vitamin D<sub>3</sub> 0.02, alpha-tocopherol (free) 25, alpha-tocopherol acetate 25, and 2-methyl-1,4-naphthoquinone 1. When vitamin B<sub>12</sub> was added, it was at a level of 0.1 mg. per kg. of diet. In the fat-free diets, vitamin D<sub>3</sub> and 2-methyl-1,4-naphthoquinone were added to the diet in alcoholic solutions. Vitamins A, E and K were added to the drinking water of chicks fed fat-free diets. Supplements of DL-methionine, ranging from 0.1 to 1.5 per cent of the diet, were added to diets containing each level of fat.

Chicks that received no vitamin B<sub>12</sub> and no supplemental methionine lost weight as the fat content of the diet was increased. When vitamin B<sub>12</sub> was added, growth was equally good with either 0, 4 or 24 per cent fat in the diet. With 4 or 24 per cent fat, the addition of methionine at 0.3 and 1 per cent raised the growth rate of vitamin B<sub>12</sub>-deficient chicks to equal that obtained in chicks receiving vitamin B<sub>12</sub>. The 1.5 per cent level of methionine was toxic in both fat-containing diets, irrespective of vitamin B<sub>12</sub> status. Moreover, with both diets, 1 per cent of methionine plus vitamin B<sub>12</sub> improved the growth rate over that obtained

with the vitamin alone. Thus methionine could not completely replace vitamin B<sub>12</sub>.

The authors emphasize the effect of supplemental methionine in the absence of fat. Methionine did not replace or spare vitamin B<sub>12</sub>; in fact the 1 per cent level of methionine had an adverse effect upon feathering, leg bone formation and growth. The wing feathers broke very easily, pigmentation was deficient and feathering on the back, breasts and legs was retarded. Vitamin B<sub>12</sub>, however, partially protected against these defects. The authors are of the opinion that the toxicity of methionine in the fat-free diets may be related to a possible role of methionine in the utilization of fat.

The effects of dietary fat level, methionine supplements and vitamin B<sub>12</sub> upon food and energy intake were also assayed. Vitamin B<sub>12</sub>-deficient chicks, unsupplemented with methionine, utilized the diet with equally poor efficiency at each level of fat intake. Thus chicks receiving 24 per cent fat had a considerably higher caloric intake per unit gain. Chicks receiving vitamin B<sub>12</sub> or methionine utilized the diet efficiently at each level of fat intake and the food intake was lowered so that the caloric intake remained relatively more constant.

No clear cut effect of the various diets could be seen upon the concentration of total fat, phospholipid and total cholesterol in the sera of the various groups of chicks cited above. The livers appeared normal and had normal concentrations of total lipids and phospholipids. The authors conclude that if derangement in fat utilization is involved in producing a vitamin B<sub>12</sub> deficiency in chicks fed the high-fat diet, more sensitive parameters of response are required to detect it.

The authors suggest these possible interrelationships between vitamin B<sub>12</sub>, methionine, fat utilization and protein synthesis. The important relationship between vitamin B<sub>12</sub> and methionine may involve synthesis or transfer of a methyl group from some source other than choline or betaine or both. Also, if vitamin B<sub>12</sub> does function in protein syn-

thesis, then the effect of vitamin B<sub>12</sub> in the high fat diets could be attributed to more efficient, but non-specific, utilization of the available methionine.

Further study is required to determine the role of methionine and vitamin B<sub>12</sub> in fat metabolism. This may be related to their ascribed role in protein synthesis.

### A STUDY OF NAIL GROWTH

*Since the normal growth rate of nails of rats and man is delayed by protein deficiency and other factors, measurement of nail growth may provide a useful index.*

Man has employed both commonplace and ingenious methods for measuring the effects of dietary substances on the body. Among the less common is the measurement of the rate of growth of fingernails. W. B. Bean (*J. Invest. Dermat.* **20**, 27 (1953)) studied the rate of growth of his own nails over a period of ten years. He used a simple method; scoring the nail at the cuticular margin to determine the length of time required for the mark to grow out to the free edge. Factors which affected rate of growth were obscure except for retardation subsequent to mumps.

Others have employed more elaborate methods for studying nail growth. W. E. LeGros Clark and L. H. D. Buxton (*Brit. J. Dermat.* **50**, 221 (1938)) scored a mark at the margin of the lunula and measured the rate of its progression periodically. By this means they found that nails grew faster in warm weather than in cool, and grew quite rapidly in nail biters. Retardation of growth was observed in poorly nourished children by M. L. Gilchrist and L. H. D. Buxton (*J. Anat.* **73**, 575 (1939)).

The rate of growth of fingernails in man has been estimated by these various authors to be between 0.085 and 0.125 mm. per day, the slower rates occurring in cool weather or under conditions of poor nutrition. M. J. Babcock (*J. Nutrition* **55**, 323 (1955)) evaluated a number of methods for measurement of nail growth and selected a technique by which migration of a scratch away from the lunula could be photographed and measured under standard conditions. Unfortunately a

few persons do not have sharply defined lunulae.

In a study of nail growth in rats, K. O. Godwin (*J. Nutrition* **69**, 121 (1959)) employed this technique and others before he selected one. He made a tattoo near the margin of the cuticle using a needle and Evans blue dye. At the same time, he made a shallow cut in the nail adjacent to the cuticular margin. Readings were made at intervals of two to three days with a filar ocular micrometer, and the average daily rate of nail growth was calculated. By this means he could measure sudden changes such as the effect of thermal or emotional stimuli (fright due to close restraint).

Changes in the diet or the environment were found to have an effect on the rat's nail growth similar to those which had been observed in humans. The mean rate of growth observed during feeding of a stock diet (18 per cent protein) was 0.106 mm. per 24 hours. By contrast, animals fed a protein-free diet had a rate of only 0.045 mm. per 24 hours. The dietary protein was increased by increments in successive groups of animals, employing a number of animal and vegetable foods. A significant correlation was found between the net dietary protein value and rate of nail growth.

Comparisons were made between the effects of adequate and inadequate amounts of vitamin A in the diets. It was found that nail growth was retarded significantly by a lack of vitamin A.

Studies also were made of the effects of exposure to cold (6°C) on nail growth. The

rate was reduced sharply regardless of whether the animals were acclimatized or not. Similarly, 5 mg. of cortisone acetate per day reduced nail growth by about half.

Of great interest is the observation that rats fed a diet containing 10 per cent raw *Phaseolus vulgaris* (haricot or snap beans) had a complete cessation of nail growth. This legume has been reported to contain a phytohemagglutinin (D. A. Rigas and E. E. Osgood, *J. Biol. Chem.* **212**, 607 (1955)). Although not considered toxic in the quantities eaten by man, this substance, which is a mixture of a mucoprotein and a euglobulin, is capable, in dilute solutions, of agglutinating erythrocytes. The nutritive value of *P. vulgaris* and of a number of other legumes has been studied with regard to the inadequacy of their proteins due to lack of methionine. The addition of this amino acid resulted in improved growth rates of rats (W. C. Russell *et al.*, *J. Nutrition* **32**, 313 (1946); *Nutrition Reviews* **5**, 144 (1947)), suggesting that the beans were deficient rather than toxic.

Unfortunately Godwin did not add methionine to the diets containing *P. vulgaris* to determine whether it would restore nail growth. He did mention a toxic factor in *P. vulgaris*, but gave no references.

Godwin's techniques and observations are of sufficient interest to workers in nutrition to warrant more than a casual glance. Most dietary studies employ "gain of body weight" in growing animals, or nitrogen balance studies in adult animals or people, to evaluate adequacy of a diet or toxicity of a drug. However, these methods are slow and reflect changes over a period of several days or weeks. If the work of Godwin can be confirmed, then rates of nail growth may provide a day-to-day index.

Nevertheless, much further work will be needed to establish parameters of nail growth under all conditions, and to determine those nutritional factors which regulate this growth. It may be that people and animals have a convenient built-in gauge by which we can read their nutritional status.

### AMINO ACID IMBALANCE, I

*The growth depression caused by addition of a mixture of amino acids creating an imbalance depends on the rate of gain supported by the original diet.*

Although there is some difference of opinion regarding the use of the term amino acid imbalance, A. E. Harper (*J. Nutrition* **68**, 405 (1959)) has defined this as any change in the proportions of the amino acids in a diet resulting in "an adverse effect which can be prevented by supplementing the diet with a relatively small amount of the most limiting amino acid or acids." According to Harper, this leaves open the question of whether there are different types of amino acid imbalances, *i.e.*, whether imbalances caused by adding relatively small amounts of one or two amino acids to a diet are identical with those produced by adding a relatively large quantity of a protein or of

an amino acid mixture lacking a single amino acid. It excludes, however, those conditions described as antagonisms and toxicities in which adverse effects are caused by the addition of a fairly large excess of a single amino acid and which are not known to be prevented by a relatively small supplement of the amino acid that is most limiting for growth.

As the result of preliminary experiments on this subject, Harper has developed an hypothesis which appears to explain why dietary additions that cause quite severe imbalances in low protein diets are almost without effect when the protein level is sufficiently high to satisfy the amino acid

requirements of the experimental subjects. The hypothesis is based on the fact that the growth response to a given increment of the amino acid that is most limiting in a diet diminishes when the growth rate becomes maximal. It follows that if the requirement for the limiting amino acid is increased by a constant amount when a quantity of an amino acid mixture causing an imbalance is added to a diet, then the growth-retarding effect of this addition should diminish as the dietary level of protein approaches adequacy.

Harper tested this hypothesis experimentally by determining the growth rates of animals ingesting diets containing: (1) a constant level of balanced protein but increasing increments of unbalanced protein, (2) a constant level of an unbalanced protein or amino acid mixture but increasing increments of balanced protein, and (3) either of the above proteins with increments of the amino acid most limiting for growth.

Groups of five male weanling rats of the Sprague-Dawley strain were fed the basal diet for three days and were then given the experimental diets *ad libitum* for a two-week period. The percentage composition of the basal diet was as follows: casein, 6.0; gelatinized corn starch, 83.6; corn oil, 4.5; mineral mixture, 5.0; choline chloride, 0.15; fat soluble vitamin mixture in corn oil, 0.5; and water soluble vitamin mixture in sucrose, 0.25.

It was found that the growth of rats fed a diet containing 6 per cent of casein supplemented with methionine was stimulated by the addition of threonine. Gelatin, which contains threonine but not tryptophan, stimulated growth when added at a level of only 3 per cent. However, when increments of gelatin greater than 3 per cent were added, the rate of gain fell off until, with the addition of 12 per cent of gelatin, the rate of gain was considerably less than that of the group fed only the basal diet. Addition of tryptophan, the most limiting amino acid,

not only prevented this growth retardation but stimulated growth above that obtained with the basal diet.

When a 3.45 per cent mixture of amino acids lacking threonine was added to diets containing casein supplemented with methionine, the greatest growth depression occurred when the diet contained 6 per cent of casein. The depression was less when the casein content was decreased to 4 per cent and very little growth depression was observed when the diet contained 15 per cent of casein.

The effect of 4 per cent of an amino acid mixture lacking threonine on the need for threonine by rats fed a diet containing 6 per cent of casein supplemented with 0.3 per cent of DL-methionine was assayed, with the result that somewhere between 0.025 and 0.05 per cent of L-threonine had to be added to overcome the growth depression.

Harper further points out that "if an amino acid mixture causing an imbalance increases the need of the animal for the limiting amino acid by a constant percentage regardless of the original level of protein in the diet, then, although the magnitude of the growth depression caused by adding a stated amount of an unbalanced amino acid mixture to a diet would depend upon the adequacy of the diet, the growth depression should be prevented by the same level of the limiting amino acid in each case."

This reasoning provided the basis for a final experiment in which three levels of casein (4, 7 and 10 per cent) supplemented with 0.3 per cent of DL-methionine in each case were fed. The growth depression was greatest when the diet contained 7 per cent of casein and was less if the diet contained either 4 or 10 per cent of casein. However, regardless of the level of casein, the growth depression was not prevented by the addition of 0.05 per cent of DL-threonine but was completely prevented by an addition of 0.1 per cent. These results are in accord with the proposed theory.

## LACTOSE AND CALCIUM ABSORPTION

*It appears that the enhancement of calcium absorption by the presence of lactose in the diet is due to a direct action of lactose either on the gut wall or within the intestinal lumen.*

The stimulating effect of dietary lactose on calcium absorption has been known for many years. A publication by F. W. Lengemann *et al.* (*J. Nutrition* **61**, 571 (1957)) has re-emphasized the favorable effect of milk on calcium and strontium absorption. The metabolism of lactose per se has been reviewed by R. L. Atkinson *et al.* (*J. Dairy Sci.* **40**, 1114 (1957)) and others. However, the mechanism by which lactose acts is still obscure.

One of the most popular theories, based upon the fermentation of the poorly absorbed lactose by intestinal bacteria and the subsequent favorable effect of an increased acidity on calcium absorption, has been challenged by P. Fournier *et al.* (*J. Physiol. (Paris)* **47**, 351 (1955)) and by R. H. Wasserman *et al.* (*J. Nutrition* **62**, 367 (1957)). Fournier and associates have shown that many of the sugars that increase calcium retention are those that are attacked with difficulty by intestinal bacteria. Wasserman's argument is based on the observation that chicks do not respond to lactose-containing milk powder whereas rats do show enhancement of calcium absorption. Both of these species have the potential for forming an intestinal bacterial population capable of fermenting glucose rapidly.

Other theories have been proffered as well. Some suggest that lactose acts by diminishing the endogenous secretion of calcium into the intestine, and others that lactose functions as a structural entity to favor bone cell metabolism.

Lengemann, Wasserman and C. L. Comar (*J. Nutrition* **68**, 443 (1959)) have recently pooled their efforts to obtain basic information on the manner in which calcium and strontium pass across the gastrointestinal barrier and how this movement is enhanced

by lactose. Calcium absorption from the gastrointestinal tract was determined by measuring the uptake of  $\text{Ca}^{45}$ ,  $\text{Sr}^{85}$ , or both, by femurs of rats after a single oral dose of a solution containing these radioisotopes.

An initial study was designed to indicate whether the lactose effect was dependent upon the type of calcium salt presented. It was found that stimulation of absorption occurred whether the calcium was present as the chloride, gluconate, lactate or acetate salt. The authors point out that this is in contrast with the data of E. Roberts and A. A. Christman (*J. Biol. Chem.* **145**, 267 (1942)), who reported that lactose had no effect on the absorption of the lactate salt in the same species. When the strong chelating agent sodium ethylenediaminetetraacetate was employed, no effect was found on normal  $\text{Sr}^{85}$  absorption, but at high concentrations it suppressed completely the action of lactose.

It thus seems that lactose acts only on ionized or readily ionizable calcium or strontium. Solubilization does not appear to be a factor, since ethylenediaminetetraacetate chelates are quite soluble. The similarity in rate of absorption in cases where the  $\text{Sr}^{85}$  is presented alone and where ethylenediaminetetraacetate is added may represent that component of the total absorption mechanism of alkaline earths caused by a diffusion-like mechanism.

To determine a possible effect of ethylenediaminetetraacetate on movement of calcium between the blood and the skeleton, the animals in two groups were given  $\text{Sr}^{85}$  intraperitoneally rather than orally. There was no effect on bone deposition of  $\text{Sr}^{85}$  in the group to which lactose had been administered.

When the absorption of  $\text{Sr}^{85}$  was deter-

mined as a function of time following oral administration, it was observed that lactose exerted its effect quite rapidly. In as short a time as two hours following ingestion, the femurs of the lactose-treated rats contained more  $\text{Sr}^{85}$  than did those of the control animals. Surprisingly, when the stomachs of the rats were excised to determine the influence of lactose on gastric emptying time, it was found that the presence of lactose resulted in a decreased rate of removal of  $\text{Sr}^{85}$  from the stomach. At 30 minutes, 80 versus 90 per cent of the activity in the respective groups had left the stomach.

The authors are of the opinion that this slowing of the rate at which the substance reaches the site of absorption cannot alone explain any enhancing effect of lactose on the extent of calcium absorption.

In one experiment, solutions were injected into ligated segments of the gastrointestinal tract. Lactose enhanced absorption most effectively in the ileum (a 4-fold increase) but an appreciable response was seen in the duodenum (a 1.3-fold increase) and the jejunum (a 1.4-fold increase). In the ileum a lactose effect on the femur was apparent at 30 minutes. However, the difference between lactose-injected and control rats was only barely significant at this time interval. The difference at one hour was much more marked, and at two hours the differences were highly significant.

It is well known that calcium is readily absorbed from the upper portions of the small intestine and less efficiently from the ileum. Since the total effect of lactose in increasing calcium absorption is about a factor of 2.0 and the relative enhanced absorption from the segments of upper intestine was about 1.4, in contrast to 4.0 for the ileum, the authors reason that the main anatomical site of lactose action is the ileum. This means that the calcium absorbed by the ileum and acted upon by lactose is that

which has escaped absorption in the upper portions.

The authors point out that the ileum has been suggested as the important site for the primary action of vitamin D in the absorption of calcium. Although vitamin D-deficient chicks and rabbits failed to respond to lactose, groups of rachitic rats showed increased absorption in the presence or absence of vitamin D when lactose was given. Apparently there is a species difference, and it would be of interest to investigate further the metabolic differences in the ileums of these species and the precise relationship between vitamin D and lactose.

The authors believe that these results tend to indicate that lactose does not act by providing substrates for acid fermentation by bacteria. In the first place, the time for the appearance of an increase in calcium absorption due to lactose seems too short for a significant amount of sugar fermentation to have occurred. Secondly, the rats had been raised on a lactose-free ration. Thus, it seems unlikely that they would possess an intestinal flora that readily attacks lactose. In addition, when the authors gave a diet containing 1 per cent of sulfadiazine for one week, they nevertheless observed an enhancing effect of lactose.

From these experiments, no definitive statements can be made concerning the exact mechanism by which lactose acts on the calcium transport mechanism. The conditions were such that lactose could not have elicited the observed response through an effect on bone. The ratio between femur  $\text{Sr}^{85}$  and absorbed  $\text{Sr}^{85}$  was not altered appreciably in the presence of lactose. Moreover, the manner by which bone-seeking radioisotopes enter the skeleton (*i.e.*, by an ion exchange and adsorption phenomena) discounts any skeletal effect of lactose. Hence the authors conclude that lactose has a direct action either on the gut wall or within the intestinal lumen.



## STARVATION AND BODY ELECTROLYTES

*Serum and carcass electrolyte concentrations in rats following acute or semi-starvation depend on the extent of weight loss. Severe weight loss results in lowered plasma potassium and an increase in carcass chloride concentrations.*

In an effort to determine the metabolic changes associated with hypokalemia and hyponatremia seen in anorexia patients (*Nutrition Reviews* 18, 71 (1960)), J. R. Elkinton and E. M. Widdowson (*Metabolism* 8, 404 (1959)) subjected groups of adult rats (body weights ranged from 238 to 355 g.) to a restricted intake of a stock diet. These rats lost 4 per cent of their body weight each week. At the end of seven weeks, when the body weight loss amounted to 30 per cent, four of the rats were killed. The serum from these animals showed no changes in chloride, sodium, potassium, calcium or magnesium concentrations when compared with the ad libitum-fed controls.

Another group of four rats that had lost 30 per cent of their body weight were given 400 mg. of sodium chloride each day during the seventh week. At the end of that period, the concentration of electrolytes in their plasma was also normal. Magnesium hydroxide feeding under similar circumstances (120 mg. per day) also had no effect on the level of plasma electrolytes.

When the rats were maintained on the restricted food intake for a total of 13 weeks, by which time they had lost 44 per cent of their body weight, the plasma potassium was reduced from 4.7 to 2.9 mEq per liter in the ad libitum-fed controls. The levels of other plasma electrolytes in these rats were normal.

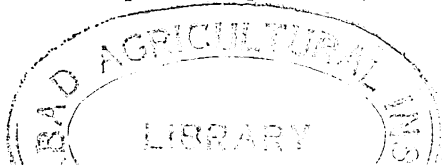
Analyses of the carcasses of the rats that had been semi-starved for seven and 13 weeks showed a reduction in the potassium concentration. The loss of carcass potassium was greater than that of nitrogen as shown by a progressive reduction in the potassium to nitrogen ratio from 2.43 to 2.27 to 1.96 in the rats semi-starved for zero, seven and

13 weeks respectively. Associated with this reduction in potassium was an increase in carcass chloride concentration, which was 30.8 mEq per kg. of fat-free wet tissue in the control rats but which increased to 37.0 mEq in the rats semi-starved seven weeks and to 40.7 mEq in those semi-starved thirteen weeks. An even greater percentage increase in chloride concentration occurred in the skin. The values for the three groups were 62.0, 77.7 and 90.3 mEq per kg. of fat-free skin respectively. The concentration of sodium tended to follow the chloride levels in the carcass and skin, but the changes were considerably smaller.

There was no significant increase in the concentration of sodium in the muscles of the semi-starved rats, whereas the chloride concentration did increase. This observation suggested to Elkinton and Widdowson the possibility that in the skeletal muscle of starved rats, chloride is sequestered in a manner which prevents the establishment of an equilibrium with the plasma chloride.

The rats that had been semi-starved for six weeks and then given large amounts of sodium chloride on each of seven days, showed no increase in body water when compared with the rats that were semi-starved without the salt loading. Thus in this respect the rat differs very markedly from semi-starved human beings, who retain water when salt is given and may go on to develop visible edema (A. Keys *et al.*, *The Biology of Human Starvation*, vol. 2, p. 921. Univ. of Minnesota Press (1951)).

E. J. Huth and Elkinton (*Am. J. Physiol.* 196, 299 (1959)) extended the above studies to seven adult rats that had been deprived of all food for three to six days (although water consumption was permitted). As a re-





sult of the starvation, the rats lost approximately 32 per cent of their original weight. The six control rats killed at the start of the experiment had 10.2 per cent fat in their carcasses, whereas the starved rats had 1.3 per cent. In spite of the marked loss of body weight and fat, the composition of the lean body mass in both the control and starved rats was essentially the same with respect to nitrogen (3.7 and 3.8 per cent respectively) and water (72.1 and 71.2 per cent respectively).

Serum collected from the starved rats just prior to the end of the experiment had the same concentration of sodium, chloride and carbon dioxide as the control animals. The potassium level was slightly lower in the serum of the starved rats than in the controls (3.9 versus 4.9 mEq per liter respectively).

Carcass analyses showed that the concentration of chloride was significantly higher in the starved rats than in the controls (42.6 mEq versus 36.6 mEq per kg. of fat-free wet tissue), while the concentration of sodium was only slightly increased in the starved rats (59.7 versus 54.7 mEq respectively). Potassium concentration, on the other hand, was slightly reduced in the bodies of the rats that had been starved (75.1 versus 81.6 mEq). These changes are similar to those seen by Elkinton and Widdowson (*loc. cit.*) in their semi-starved rats.

Samples of muscle removed from the thighs of the starved rats showed a significant increase in the chloride concentration (16.0 for the starved rats versus 13.8 mEq per kg. of fat-free wet muscle for the controls). Associated therewith was an increase in the extracellular water from 123 g. per kg. of fat-free muscle in the controls to 139 g. in the starved rats. Calculations for these extracellular fluid volumes were based on the assumption that the extracellular fluid is equivalent to the chloride space. The concentrations of sodium, potassium and nitrogen in the thigh muscle showed no alteration during starvation.

The above data suggest that in acute starvation resulting from the complete absence of food, the extracellular fluid appears to increase at the expense of the intracellular. Huth and Elkinton explain the difference between the electrolyte changes in the thigh muscle and those in the fat-free carcass as caused by the higher proportion of non-metabolizable solids in the carcass. This resulted in a decrease in the water content of the fat-free carcass, while that of the fat-free skeletal muscle increased.

An extensive study of acute and semi-starvation in rats was made by E. M. Widdowson and R. A. McCance (*Brit. J. Nutrition* 10, 363 (1956)). The authors were primarily interested in determining whether the two conditions produced similar biochemical changes. The frequent occurrence of edema in chronic or semi-starvation and its absence in individuals undergoing acute starvation is the most prominent difference between these two conditions observed in human beings.

These British investigators fed a nutritionally adequate diet composed of natural foods to six adult male (315 g. body weight) and six female (220 g. body weight) rats. The food intake of these animals was restricted so that their body weights at the end of six weeks would be the same as that of animals fed the same diet on an ad libitum basis for five weeks and then subjected to six days without food. A control group fed the same diet on an ad libitum basis for five and one half weeks provided the base line data. The latter group showed only a slight increase in body weight, whereas the other two groups lost 15 per cent of their body weights.

Carcass analyses showed that the animals whose food intake had been restricted for the six-week period were similar to those that had been without food for the last six days of the experiment. This was true for protein, sodium, potassium, calcium, magnesium, chloride and phosphorus. There were slight differences for some of these elements

between the semi- and acutely-starved rats, but these differences did not appear significant and were not consistent for the sexes. In most cases, the reduction in the concentration of these electrolytes was the same as the reduction in body weight.

A possible explanation for the failure of the rats starved by Widdowson and McCance to show significant changes in potassium may be the relatively small percentage of body weight lost by their rats. Although the initial weights of the rats used by both groups of investigators were essentially the same, the weight loss of the rats used in the British study was only 15 per cent, while in the American study it was 32 per cent. Moreover, there were marked differences in the percentages of body fat lost. The rats

starved by Widdowson and McCance lost, at the most, 40 per cent of their fat stores, while the rats starved by Huth and Elkinton lost 90 per cent of their fat stores.

It is possible that the disturbances in potassium metabolism occur only when the body weight and body fat losses become of considerable magnitude. This would be consistent with the results of studies with anorexia nervosa patients, in whom changes in serum electrolytes were observed only when the body weight loss exceeded 25 per cent (*Nutrition Reviews* 18, 71 (1960)). Nevertheless, while some of the results with rats parallel the alterations in potassium metabolism seen in anorexia nervosa patients, it remains for future work to elucidate the mechanisms that bring about these conditions.

### NUTRITIVE VALUE OF FRYING OILS

*Although at least three types of toxic materials may be produced by overheating or oxidizing oils, hydrogenated frying oils used commercially may retain their nutritive value.*

Interest in the possible deleterious effects of overheated fats has continued unabated for over 20 years (see, for example, *Nutrition Reviews* 2, 18 (1944); G. O. Burr and R. H. Barnes, *Physiol. Rev.* 23, 256 (1943)). However, enthusiasm has not generally been matched by accomplishment and, with few exceptions, little real progress can be claimed.

There is still some disagreement, for example, concerning the types of compounds responsible for the toxic effects of the overheated fats, with some research workers claiming peroxides as the sole toxic agents and others placing the entire blame on polymeric products (*Nutrition Reviews* 14, 28, 122 (1956); 15, 346 (1957)). From a perusal of the literature, however, it seems obvious that both types of products are toxic, the peroxides being produced largely under conditions of moderate temperature in the presence of air, while the polymeric type

products (probably cyclic monomers) are produced under high-temperature anaerobic conditions.

Nevertheless, two questions of primary importance have not been answered at all. First, what is the damaging mechanism in both types of toxic agents and, second, what are the amounts of such agents actually formed in fats used in standard cooking processes?

In a recent paper, E. G. Perkins and F. A. Kummerow (*J. Nutrition* 68, 101 (1959)) reported an investigation of the nutritive properties of corn oil stirred with air at 200°C for 48 hours. This thermally oxidized oil was converted to the fatty acids, which were separated by urea fractionation into an adduct-forming portion (64 per cent, probably straight-chain acids) and a non-urea-adduct-forming portion (36 per cent, probably polyunsaturated and branched or cyclic acids or polymeric products). The latter fraction was subjected to molecular distillation at 1 to 3

microns pressure and 150°C, thus separating monomeric from polymeric fractions.

All fractions were fed to weanling rats as 12 per cent of a diet consisting of 50 per cent glucose, 31 per cent casein, 2 per cent additional fat (fresh cottonseed oil), 5 per cent salt mixture and adequate supplements of water- and fat-soluble vitamins.

The rats on the non-distillable, non-urea-adduct-forming fraction lost weight and died within seven days. Those on fresh corn oil fatty acids, the urea-adduct-forming fraction or the molecular distillate of the non-urea-adduct-forming fraction all gained weight normally and appeared in good health. Growth was also severely depressed by the total thermally oxidized fatty acids or the total non-urea-adduct-forming fraction. Dilution of the latter with an equal weight of fresh corn oil fatty acids alleviated, but did not prevent, the growth-inhibiting action.

The authors suggest that the toxic fraction, which represents 30.6 per cent of the original oil, is a relatively small proportion and that most of the oil is undamaged by the treatment. It is possible that the toxic material is actually present in considerably smaller amounts since it has been reported previously that the actual polymeric fractions formed during anaerobic heating to 275°C are not very toxic (*Nutrition Reviews* 15, 346 (1957)). In this case, therefore, only part of the polymers may be responsible for the effects.

It might also be proposed that not two but three distinct toxic products are produced by different treatment of the oil. First, at high temperatures in the absence of oxygen, the cyclic monomer is the major toxic substance. On the other hand, hydroperoxides are the major toxic substance after treatment at moderate temperatures for short duration in the presence of oxygen. Moreover, it has been shown in the present work that at high temperatures in the presence of oxygen, toxic oxygen-containing polymers are produced.

The authors do not state that the condi-

tions employed in these studies duplicate those of actual frying processes, but they point out that caution should be exercised in the dietary use of unsaturated oils which have been subjected to such treatment.

In a study of fats actually used in frying, K. W. Keane, G. A. Jacobson and C. H. Krieger (*J. Nutrition* 68, 57 (1959)) determined the caloric value of a hydrogenated cottonseed oil which had been used for periods of 14 to 24 days in a commercial frying operation. The used or fresh oil was incorporated as 23 per cent of a basal diet composed of 20 per cent casein, 73.6 per cent sucrose, 2 per cent cottonseed oil, 4 per cent mineral mixture and adequate supplements of water- and fat-soluble vitamins.

It was found, somewhat surprisingly, that the caloric values of the oil samples, based on the caloric restriction technique described by E. E. Rice, W. D. Warner, P. E. Mode and C. E. Poling (*J. Nutrition* 61, 25 (1957)), were directly related to the length of use of the oil in the frying process. Searching for some chemical changes in the oil samples which might account for the changes in their nutritive properties, the authors found that the total non-conjugated double bonds increased in direct proportion to the caloric value. The same relationship was also shown to hold for different stages of hydrogenation of cottonseed oil.

It was evident from these experiments, therefore, as shown previously, that caloric availability of fats is related to their unsaturation (see, for example, *Present Knowledge in Nutrition*, The Nutrition Foundation, Inc., 1956). In this case, however, the mechanism of double bond production was of interest, since it is usually found that heated or aerated fats show decreases in unsaturation because of peroxidation or polymerization.

In an analysis of the alterations in double bonds during 18 days' use of the fat, it was found that there was an increase in dienoic and possibly conjugated dienoic acids, but little change in any of the other polyunsaturated acids. The reasons for these in-

creases, however, are uncertain since the authors intimate that there was probably a considerable contribution from the fat of the chicken parts being fried but do not include quantitative data. Moreover, fat was being replaced at an unspecified rate and no assessment was made of the contribution of this process. No logical explanation, therefore, can be given for the changes in unsaturation, particularly since the temperature and other conditions were not stated.

In a further evaluation of the used hydrogenated cottonseed oil, Keane and co-workers carried out experiments in which weanling rats were fed the basal diet with 18 per cent of variously treated samples of hydrogenated cottonseed oil with or without an additional 2 or 4 per cent cottonseed oil as a source of essential fatty acids. It was found, in confirmation of the previous results, that growth rate for five weeks was greatest for the used hydrogenated cottonseed oil. Moreover, the additional cottonseed oil gave greater growth rate only if the hydrogenated oil had been "laboratory heated and oxidized" and gave a subnormal growth response. Even in this case, no symptoms of toxicity such as diarrhea or rough hair coat appeared.

When the same results were obtained with corn oil (which, however, was an old sample and gave poor growth response) the authors felt the need of explaining the differences between these findings and those of other workers, who usually obtain low caloric values and toxic symptoms with heated or oxidized oils. In a comparison of their diet

with that used by O. C. Johnson, T. Sakuragi and Kummerow (*J. Am. Oil Chem. Soc.* **33**, 433 (1956)), Keane and co-workers pointed out that their own diet contained additional cystine, glycine, inositol, niacin, para-aminobenzoic acid and biotin while these substances were lacking in the diet of Johnson and co-workers, thus possibly accounting for the toxic effect of the oxidized oil.

However, there may be other explanations for the discrepancies not readily apparent. It is to be expected that a hydrogenated oil will not suffer decomposition on heating as readily as a highly unsaturated oil. Moreover, if a fat high in linoleic acid (such as chicken fat) is being continuously added, the results will not be strictly comparable to the simple case.

The authors did not indicate what was meant by a "laboratory heated and oxidized" oil nor did they describe the properties of the product of this treatment, which might have facilitated comparison. It is certainly true that the composition of the remainder of the diet has a great deal to do with the effect of the fat, but the literature is almost universally agreed that overheated and oxidized unsaturated oils are detrimental to the well-being of animals.

The value of these studies lies in the information that an actual commercial frying operation has apparently not destroyed the nutritional value of the fat used in cooking but may even have increased it. As the authors state, there is a need for further research on this problem.

### VITAMIN B<sub>12</sub> DEFICIENCY

*A high percentage of the offspring of vitamin B<sub>12</sub>-deficient rats was hydrocephalic and showed other evidences of organ retardation at birth.*

L. R. Richardson and A. G. Hogan's experiments on inadequate maternal nutrition (*J. Nutrition* **32**, 459 (1946)) led subsequent investigators to suspect that a deficiency of

either folic acid, vitamin B<sub>12</sub>, or both was responsible for the resulting hydrocephalus in infant rats. Although B. L. O'Dell, J. R. Whitley and Hogan (*Proc. Soc. Exp. Biol.*

*Med.* 69, 272 (1948)) demonstrated that a deficiency of folic acid was the chief cause of the congenital abnormality, it was later found (O'Dell, Whitley and Hogan, *Ibid.*, 76, 349 (1951)) that a high proportion of offspring are afflicted with abnormalities if the mothers are severely depleted of vitamin B<sub>12</sub>.

P. M. Newberne and O'Dell (*J. Nutrition* 68, 343 (1959)) have described in considerable detail the pathology of the central nervous system of vitamin B<sub>12</sub>-deficient rat embryos and have included observations on the peripheral nerves, lungs, kidneys and adrenal glands from vitamin B<sub>12</sub>-deficient and control embryos.

To deplete the body stores of vitamin B<sub>12</sub>, female rats were fed, from weaning, a diet of the following composition: soybean oil meal (70 per cent), glucose (22 per cent), lard (4 per cent), salts (4 per cent). Vitamins given (in milligrams per 100 g. of diet) were: thiamine hydrochloride, 1.6; riboflavin, 1.6; pyridoxine hydrochloride, 1.6; calcium pantothenate, 4.0; choline chloride, 100; biotin, 0.02; folacin, 0.5; alpha-tocopherol, 3.0; 2-methyl-1,4-naphthoquinone, 1.0; vitamin A, 2000 I.U., and vitamin D, 280 I.U. The control animals were fed the basal diet supplemented with 30 mg. of vitamin B<sub>12</sub> per kilogram of diet. The criteria used to determine the deficiency state were a high incidence of hydrocephalus and a mortality of 80 to 90 per cent during the first week.

In the hydrocephalic newborn rat the parenchyma of the brain was spongy and areolar with many distended spaces. The neurons were shrunken and nuclei showed vacuolation. Cytoplasmic changes ranged from slight loss of Nissl substance to gross chromatolysis. The glia showed some proliferation of oligodendroglia which, together with the astrocytes, appeared swollen and vacuolated with degenerative changes in some cases. Using special stains, the authors were able to demonstrate a complete lack of glycogen in the brain of experimental animals as compared to considerable amounts in the choroid plexi and connective tissues of controls.

Adrenal glands were consistently smaller in the experimental animals than in controls of the same body weight. The capsule was thin while the zona arcuata was more pronounced than normal, apparently because of super-staining pyknotic nuclei in the deficient animal. Nuclei of the fascicular zone were pale and the cytoplasm of these cells was greatly distended with lipid, but the adrenal medulla showed no significant deviation from the normal.

The lungs of the experimental offspring typically showed delayed development with poor differentiation into airspaces and considerable distance between the capillaries and their alveolar walls. However, the kidneys exhibited more retardation of growth than any other organ. The embryonic-like cortex consisted mainly of poorly differentiated glomeruli and tubules, the proximal ones being more mature but grossly dilated. C. C. Jones *et al.* (*Proc. Soc. Exp. Biol. Med.* 90, 135 (1955)) has made similar observations.

Although myelination of the central nervous system did not seem to be affected by the state of deficiency, the spinal cord consistently showed areas without myelin and the more peripheral nerves revealed either a complete absence or very little myelination of the fibers. The Schwann cells of the myelin sheath appeared to be more numerous in the B<sub>12</sub>-deficient animals, but fibrosis was not observed.

Histological study of embryonic brains of rats of different ages revealed no discernible changes in the brains of 14-day embryos as a result of maternal deficiency of vitamin B<sub>12</sub>. By the sixteenth day of gestation approximately 25 per cent of the embryos showed some degree of hydrocephalus with marked alterations in the aqueduct. The most important finding was a larger number of mitotic figures in the aqueductal ependyma of the deficient brains on the sixteenth day of gestation. This is evidence that the abnormal histological pattern developed in

the deficient brain sometime between the fourteenth and sixteenth day of gestation.

The actual hydrocephalus resulting from a vitamin B<sub>12</sub> deficiency appears to be caused primarily by constriction or closure of the cerebral aqueduct. The authors suggest that the greater number of mitotic figures in the

aqueductal area in the 16-day embryonic brain indicates that mitosis is slowed in this particular area (the cells are perhaps held in states of arrested mitosis), and that continued growth of near normal brain tissue constricts the aqueduct and thus precipitates hydrocephalus.

### THE PROTEIN SYNTHESIS MECHANISM IN THE PROTEIN-DEPLETED DOG

*Methionine labeled with S<sup>35</sup> was injected into normal and protein-depleted dogs. With depletion increasing to a loss of 25 per cent of body nitrogen, plasma proteins showed increasing radioactivity.*

Nitrogen equilibrium can be maintained in an adult dog with 250 mg. of nitrogen as casein and 80 calories per kg. body weight per day (J. B. Allison, J. A. Anderson and R. D. Seeley, *Ann. N. Y. Acad. Sci.* **47**, 245 (1946)). Isocaloric replacement of protein with carbohydrate does not eliminate loss of nitrogen in the urine but the rate of loss becomes less. The urinary nitrogen falls to a minimum of about 60 mg. per kg. by the sixth week of depletion. As the catabolism of body protein continues, the animal becomes weak, anorexic and edematous. Unless refeeding is done early enough the dog may die in spite of any measures.

Depletion may cause irreversible damage to the protein synthetic mechanism (Allison, *Symposia on Nutrition*, vol. 2, p. 123. C. C. Thomas, 1950). Similar irreversible damage to protein metabolism may sometimes be seen in man.

Kwashiorkor is a clinical syndrome found in human infants in many tropical countries. After finishing breast feeding, infants are often transferred to diets adequate in calories but poor in protein. In some instances the resulting protein depletion may continue too long to be reversible by protein and electrolyte repletion.

In relation to this problem, J. S. Garrow (*J. Clin. Invest.* **38**, 1241 (1959)) has reported experiments attempting to correlate the degree of protein-depletion with changes

in protein metabolism detectable with isotopic tracers.

Eight clinically normal adult dogs were maintained in nitrogen equilibrium on a synthetic diet containing 250 mg. nitrogen (as casein) and 80 calories per kg. body weight per day. Each was injected intravenously with  $4 \times 10^6$  counts per minute in 0.5 mg. S<sup>35</sup> methionine and 4 mg. of T-1824 dye per kg. body weight. Four received labeled DL-methionine and four received labeled L-methionine. Two animals in each group of four were started on a protein-free diet on the day of injection and two were started one week later.

During depletion the dogs were reinjected with T-1824 and S<sup>35</sup> methionine after intervals of three to ten weeks. Four of the dogs were injected a third time after a similar interval. When bleeding was used to accelerate depletion, enough time was allowed between bleeding and injection to permit equilibration of the protein pools.

The cumulative loss of nitrogen was measured in daily urine and weekly feces collections and in blood collected. The dye concentration in plasma was measured in samples taken one-quarter hour and one hour after injection. Plasma volume was measured by extrapolation to zero time.

Radioactivity measurements were made on precipitated plasma proteins and the supernatant. After precipitating the proteins



with trichloroacetic acid they were redissolved in sodium hydroxide and sampled for radioactivity determinations. When reinjections were done, the preinjection residual activity was subtracted from the subsequent values determined after injection. Radioactivity from  $S^{35}$  was expressed as specific activity per mg. nitrogen. If one assumes that the sulfur to nitrogen ratio in protein is constant, the figure is proportional to the true specific activity.

The depletion program lasting from 50 to 60 days resulted in a loss of 30 per cent of body nitrogen. Body weight lost ranged from 13 to 18 per cent. The fact that this value is so much less than the per cent loss of nitrogen indicates that some nitrogen-poor component is being retained, *e.g.*, edema fluid. From 20 to 25 per cent of all the nitrogen lost was in the feces, and the nitrogen lost daily averaged about 0.5 per cent of the total.

During depletion of four of the dogs, plasma volume and protein concentration was measured. The circulating plasma protein was rapidly reduced in the later stages of depletion, exceeding the rate of loss of total body nitrogen.

Radioactivity from protein-free plasma fell from 4500 to 350 counts per minute per ml. during an 8-hour period after injection. There was no significant difference between groups either before or after depletion or with L- or DL-methionine.

The amount of incorporation of either L- or DL-methionine into plasma protein was the same. Curves of activity at one, two, four, six and eight hours following injection were similar. However, the height of the curve was much greater in the depleted dogs. Peak activity increased with increasing depletion. The possibility that this increase in peak activity finally reaches a maximum and then declines was suggested in a comparison of 20-per-cent-depleted animals with 32-per-cent-depleted animals. Plasma protein decay curves were not different in normal and depleted dogs. Excretion of labeled sulfur was

essentially similar in both groups, although the depleted dogs appeared to excrete the label a little more rapidly. The specific activity of the urine reflected that of plasma protein.

Two factors may account for the difference in specific activity of plasma protein with increasing depletion. One is the reduction in the size of the plasma protein pool, the other is an increased proportion of labeled molecules entering this pool. Blood volume determinations revealed that when 30 per cent of body nitrogen had been lost the circulating plasma protein was half the normal amount, although this was not enough to explain the increased specific activity of the plasma proteins. When 25 per cent of body nitrogen had been lost the greatest amount of incorporation of the radioactive dose occurred.

C. L. Yuile, F. V. Lucas, R. D. Neubecker and G. H. Whipple (*Fed. Proc.* **14**, 424 (1955)) reported that dogs depleted by plasmapheresis as well as low-protein feeding showed a greater reduction in the extravascular extracellular protein pool than the circulating plasma protein pool. Garrow, however, does not believe that reduction of the extravascular pool could account for increased activity of intravascular protein at six to eight hours after injection, since at least two days are required to equilibrate the two pools.

As a result of analytical techniques, the absolute quantity of radioactivity in intravascular protein may be expressed as per cent of original dose. In normal dogs the blood proteins account for 4.5 per cent of the injected dose. For the second and third injections the averages are 7.2 and 6.5 per cent. Thus with depletion the blood proteins represent increased amounts of the dose.

Study of the excretion of radioactivity permitted a partial evaluation of the fate of the injected label. As depletion progressed, nitrogen excretion diminished and isotope excretion increased slightly. Three assumptions were made in discussing the implica-



tions in this experiment: (a) free and protein-bound methionine reach equilibrium by the seventh day after injection; (b) all labeled sulfur not excreted has been incorporated in protein by the seventh day; and (c) other amino acids behave in a similar manner.

The results indicate that urine and plasma protein specific activities are similar and increase with depletion. Total body radioactivity in counts per minute per mg. of nitrogen resembles the activities of normal urine and plasma protein, but depleted dogs show two to three times the average body radioactivity in their urine and plasma protein.

The fact that even severely depleted dogs excrete as much as 80 mg. nitrogen per kg. body weight daily, which in a 5 kg. dog would be about 2.5 g. nitrogen per day, makes it seem unlikely that the plasma protein would be the main source since in the

dog it contains only about 10 g. of protein. This high activity of urinary nitrogen must then come from some fairly large high activity pool.

During the early stages of protein depletion readily available protein from liver and some from plasma is expended first. Later the proteins of slower turnover are used to furnish priority tissues with essential amino acids. Just as destruction of protein shifts during depletion so does the synthesis of protein shift to tissues important to the survival of the organism.

As depletion progresses the loss of important cell enzymes may result in irreversible damage to synthetic mechanisms. Thus the redistribution of anabolic potential may be a more important facet of the concept of protein depletion than the state of the protein stores.

### INFLUENCE OF BODY COMPOSITION ON SURVIVAL UNDER STRESS

*Piglets fed diets with varying amounts of fat and protein were later subjected to fatal stress (lack of food, lack of water, or both). The results may prove invaluable in planning for human survival.*

The problem of survival is of great concern to our armed forces, as well as to civilians in times of disaster or war. Aside from physical injury or exposure to extremes of heat or cold, the greatest hazard is deprivation of water, since man can live for a considerable period if deprived of food alone.

L. S. Bauer and L. J. Filer, Jr. (*J. Nutrition* 69, 128 (1959)) have studied the influence of diet upon the survival of baby pigs undergoing three types of deprivation. They selected three-day-old piglets and fed half of them a diet which was high in fat and low in protein while the other half received a diet high in protein but low in fat. All animals were allowed to eat and drink at will. At the end of eight weeks, some of the animals from each group were killed in order to determine their body composition. Those fed the high-fat, low-protein diet had more body fat

while those fed the high-protein, low-fat diet had more body water and body protein.

The remaining animals were subjected to one of three kinds of stress: complete deprivation of food and water (stress A), deprivation of water alone with dry food available (stress B), and deprivation of food alone with water available (stress C). Under stress A (complete deprivation of food and water) the pigs fed a high-protein diet lived 28 days, compared with 21 days for the group fed a high-fat diet. However, there was no difference in survival of the two groups when stress B (deprivation of water) was applied; the animals lived 15 to 16 days. By contrast, stress C (deprivation of food) resulted in a marked difference. Under these conditions, the group fed a high-fat diet lived 89 days while those fed a high-protein diet lived only 36 days.

Analysis of the carcasses of these animals

disclosed several significant findings. When deprived of food but with water available (stress C) the pigs used almost all of their body stores of fat and much of their stores of protein. However, as the fatty stores were depleted, the content of water in the body increased. Under all conditions of stress, the weight of the heart tended to increase while the weight of the spleen decreased. The liver became smaller in pigs which were starved.

Studies of electrolytes (sodium, potassium and chloride) in the carcasses of the pigs revealed that complete deprivation of food and water (stress A) caused the sodium and chloride content to rise and the potassium content to fall. Starvation with water available (stress C) resulted in the same changes in electrolyte content but to a greater degree, especially in the animals fed a high-fat diet. It must be remembered, however, that these pigs lived much longer than those under stress A.

Urine, which was collected from a few members of each group, changed in specific gravity, total volume, and in osmotic readings as follows. Those pigs deprived of water and food or of water alone voided highly concentrated urine. Those deprived of food alone excreted dilute urine, but the pigs fed the high-fat, low-protein diet excreted much less urine than did those fed the high-protein, low-fat diet.

It was surprising that pigs deprived of water would continue to eat food for ten days, even though this food aggravated their dehydration.

The following conclusions were drawn from these studies. Under conditions of both starvation and thirst, animals with larger stores of body water (high-protein diet) survived longer. However, under conditions of starvation alone, the reverse was true. This may be explained by the fact that fatty stores can provide energy for longer periods of time than can protein stores, but the latter

are accompanied by a larger reserve of water. Even though catabolism of fat results in production of some water, the quantity so produced is insignificant.

It has been believed that, under conditions of starvation, the body selectively burns fat first and catabolizes protein only as a last resort. However, the evidence presented herewith contradicts this belief, provided adequate supplies of water are available.

The authors estimated the rates of catabolism of fat and of protein for animals under each type of stress. They concluded that the daily utilization of fat was reasonably constant under all three forms of stress. However, a different situation resulted respecting protein catabolism; the average loss of protein in animals having the greatest stores of protein was two to three times greater than in those having a lesser amount under all three conditions of stress. Furthermore, protein catabolism continued as a constant rate throughout the entire period of stress, as judged by concentrations of urea in the blood. They surmised that the body, once accustomed to a high-protein intake is unable to "gear down" its metabolic processes in time to permit survival.

This study suggests that our customary high-protein diets may not be ideal to prepare us for survival under conditions of sudden deprivation. It also lends credence to the tales of shipwrecked sailors who described early death for the robust members of a crew and longer survival for their less muscular comrades (assuming water was available).

This study might have been improved had the individual groups of animals been larger and of equal size. Also, since young animals generally have a lesser capacity to conserve water and electrolytes than do adults, it might have been better (though more costly) to have used full-grown pigs. However, these are minor objections to a convincing study.

## NOTES

## Letter to the Editor

Dear Sir:

I feel that exception must be taken to the statement at the end of the first paragraph of the review "Fibrinolysis and Lipid Metabolism" (*Nutrition Reviews* 17, 263 (1959)), as it is not a statement of fact.

In the reference cited by you, Fullarton does not state that hyperlipemia may retard fibrinolysis nor do I state that hyperlipemia may accelerate blood clotting. Indeed, I was at some pains to express the point of view that it was difficult to see how acceleration of the clotting time of blood in a test tube can have a bearing on intravascular clotting.

Neither in the case of Fullarton nor in my case is your reference to our respective original work but, in the one case, to reported remarks at a conference and, in the other, to correspondence. This failure to quote original work I regard as a serious omission on your part, in view of the stated aim of *Nutrition Reviews* to make "available an unbiased, authoritative review."

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**Essential Fatty Acids, Serum  
Cholesterol and Coprophagy**

It has been known for a long time that laboratory animals can obtain several vitamins by coprophagy (*Nutrition Reviews* 7, 283 (1949)). The source of such vitamins appears to be largely the intestinal flora, which can, under certain conditions, eliminate the need for an outside source of vitamins (*Ibid.* 16, 126 (1958)). R. H. Barnes *et al.* (*J. Nutrition* 63, 489 (1957)) have developed a method for prevention of coprophagy in the rat and have applied it to a study of the ef-

fect of this practice on the requirements of various vitamins (Barnes, E. Kwong and G. Fiala, *Ibid.* 67, 599 (1959)).

Despite this clear-cut evidence that a variety of essential nutrients is obtained or at least augmented by coprophagy in several animal species, no attempt had been made to see if this applied to essential fatty acids. Thus in an extension of their previous work, Barnes, S. Tuthill, Kwong and Fiala (*J. Nutrition* 68, 121 (1959)) raised male weanling rats on a fat-free diet with or without supplementary corn oil and with or without access to their feces. Rats on both diets grew more slowly when coprophagy was prevented, and this was especially pronounced in the fat-deficient groups, in which the average difference in weight was about 50 g. Moreover, without coprophagy, fat-deficient rats developed symptoms more rapidly and extensively.

Since serum cholesterol values were found to be low in all animals on the low-fat diet, a further study was made of the effect of different dietary fats on the serum cholesterol of fat-deficient rats. Groups of weanling or young adult rats were given fat-free diets alone or with supplements of 1 per cent corn oil, 15 per cent hydrogenated coconut oil, or both. The weanling rats without supplementary corn oil grew poorly, but, as was expected, the young adults were not affected by the deficiency. In both groups, the greatest growth rates were observed in rats given both corn oil and hydrogenated coconut oil. Serum cholesterol values were lowest for the fat-deficient rats and highest in rats fed the combined corn oil and hydrogenated coconut oil supplements. Especially in the case of the weanling rats, the combined oil supplements produced higher serum cholesterol values than did hydrogenated coconut oil alone.

Although these experiments did not demonstrate how the fecal unsaturated fatty acids were derived, it is evident that they were not absorbed from the cecum or large

intestine and only had an effect if re-ingested with the feces. For rapid production of essential fatty acid deficiency, this factor will have to be taken into consideration.

The effect of the unsaturated fat in potentiating the serum cholesterol elevating effect of the saturated fat is somewhat obscure and should be investigated further. The many complex relationships between serum and tissue cholesterol concentrations and dietary fat are far from solution, and at present each new finding seems only to raise further questions.

#### **New Nutrition Journal to be Published in Germany**

*Zeitschrift für Ernährungswissenschaft-Journal of Nutritional Sciences-Journal des Sciences de la Nutrition* will shortly appear, with provision for publication of manuscripts in German, English or French. This journal will provide for a wide coverage in subject matter related to foods and nutrition, including clinical nutrition and dietetics, human, animal and plant nutrition, physiology and biochemistry, nutritional chemistry and food technology, toxicology and hygiene, agriculture and veterinary medicine, preserving, storing and transport techniques, and food statistics.

*Editor-in-Chief:* Professor Konrad Lang, Institut für Physiologische Chemie, Mainz, Germany.

*Co-editors:* E. Abramson, K. Bernhard, J. Bruggemann, H. Dam, W. Droese, A. Hock, J. Kuprianoff, W. Lenkeit, H. Malmros, R. Nicolaysen, L. Schmid, A. I. Virtanen.

#### **Diet and Celiac Syndrome**

A well documented paper on the practical dietary management of patients with celiac syndrome by E. B. Mike (*Am. J. Clin. Nutrition* 7, 463 (1959)) will be of interest to clinicians and dietitians dealing with such patients. The paper provides a tabulation of basic principles of diet for idiopathic celiac disease and for cystic fibrosis of the pancreas. It further includes a guide for the selection of foods for the diets of patients of various ages with these syndromes, based on experience at Babies Hospital, Columbia-Presbyterian Medical Center, New York City.

The paper is quite detailed, providing meal plans, dietary patterns and an outline of a low-gluten diet. It will serve not only as a handy practical guide but also a ready reference to some 40 papers which give more detailed information on the subject.

# NUTRITION REVIEWS

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## ARSENIC AND SELENIUM IN RELATION TO THE FOOD ADDITIVE LAW OF 1958

The Delaney clause in the Food Additive Amendment of 1958 (Public Law 85-929) was designed to protect the public against assumed dangers of acquiring cancer through foods. It provides "that no additive shall be deemed safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal." The law is specifically directed to chemical additives to food.

The amendment itself precludes scientific judgment in that it provides for no tolerance, even for essential nutrients, which may prove carcinogenic in huge dosage in one species or another. Certain common foods, food ingredients and products of human metabolism have been reported to induce cancer when ingested by experimental animals. The principal dietary influence reported to lessen or deter incidence of experimental cancer appears from important pioneering studies to be calorie restriction (A. Tannenbaum and H. Silverstone, *Advances in Cancer Research* 1, 451 (1953)). As clearly stated by these authors, further research is needed to establish the role of a number of naturally occurring minor constituents.

The complexity of the problem is greatly magnified by vagaries in test methods for various carcinogenic influences and the true significance of any of these for humans. The problem is also fraught with emotional aspects, even at the scientist's level, simply because too little is clearly known. Public anxiety may in turn be engendered by uncertainty and inconsistency in administration of the law. This sequence appeared inevitable following strict interpretation of the amendment (*Federal Register* 24, p. 4376 (1959)).

The question comes whether the tissues of food animals ever harbor enough of any of the approved feed additives to adversely influence other animals which consume them. Indeed, experiments are needed to determine whether the effectiveness of even potent carcinogens can be thus transmitted from animal to animal. Once we subscribe, however, in point of law to precepts based on supposition rather than measurement, the effects may be disastrous. The bondage imposed by the new law appears to bar and confuse progress in food production without accomplishing its desired goal. This is illustrated in the case of selenium, now reported both a carcinogen and a nutrient; also in the case of arsenicals whose long history in medicine and agriculture has been repeatedly confused by vague speculation and gross misinformation.

Extensive studies by the U. S. Public Health Service of orchardists around Wenatchee, Washington, provided a basis in 1941 to establish an arsenic tolerance of 3.5 p.p.m. arsenic trioxide equivalent on certain fruits (P. A. Neal *et al.*, *Pub. Health Bull. No. 267* (1941)). This provided a carefully established tolerance for residues of arsenic on fruits when lead arsenate was used as a spray. There was more concern about lead in this study, and justifiably so, than about arsenic. The same position for arsenic residues was re-established in observations for the Pesticide Chemicals Amendment of 1954. This includes tolerances for calcium arsenate as an insecticide for use on vegetables. One pertinent statement reads, "Calculated as arsenic trioxide, about 3 milligrams a day can be tolerated without hazard to man" (*Federal Register*, pp. 6760-6772 (1954)).

No evidence impugning inorganic arsenicals appeared following the above

disclosure. Nor was any implication made against organic arsenicals at the Symposium on Medicated Feeds sponsored by the Department of Health, Education and Welfare (*Medical Encyclopedia*, p. 136 (1956)). Use of inorganic arsenates decreased greatly with the advent of newer, more effective agents. Even so, it may be noted that residues of organic arsenicals in animal tissues are not now accorded the same tolerance which applies to some 16 fruits and 25 vegetables. Concern about arsenicals is natural and wise, but when properly used few other groups have served mankind better in so many ways.

Both arsenic and selenium became problems for investigation and review under the above mentioned interpretation of Public Law 85-929. Arsenic per se was incriminated as an environmental carcinogen in the older literature. There appears agreement now, however, that "arsenic" has not been shown to cause cancer in experimental animals. (*Problems in the Evaluation of Carcinogenic Hazards from Use of Food Additives. National Research Council* (1960)).

In a fervent plea for conservatism and thoroughness in epidemiologic search to find true causes of cancer, Sir Ernest Kennaway (*Lancet* 2, 769 (1942)) rejected historic evidence commonly cited against arsenic. In support of this, E. Boyland (*Nature* 159, 554 (1947)) stated: "No unequivocal evidence has ever been presented to show that arsenic is carcinogenic." Statements of the carcinogenicity of arsenic greatly outnumber the denials. Close examination of the literature reveals, however, that the concept, once started 140 years ago, was established largely by reiteration until it achieved textbook status.

Palmar and plantar keratoses and abnormal pigmentation are occasional toxic sequelae of massive arsenite therapy (L. S. Goodman and A. Gilman, *Pharmacological Basis of Therapeutics. Macmillan* (1955)). But only about 20 per cent of patients who develop these symptoms of arsenite

poisoning are also reported to develop carcinomas (O. S. Ormsby and H. Montgomery, *Diseases of the Skin. Lea and Febiger* (1954)). O. Neubauer (*Brit. J. Cancer* 1, 192 (1947)) reviewed the medical evidence, but rounded up only 143 cases of "arsenical cancer" from the world literature. All such cases are based on *a priori* evidence, without suitable controls. Many are clearly in doubt if only because of the long interval, average 18 years, between suspected treatment and the appearance of cancer. Millions of people have received inorganic arsenite therapy since its introduction by Fowler in 1786. Furthermore, such therapy was used chiefly for control of psoriasis, pemphigus, acne, dermatitis herpetiformis, lichen planus and other chronic non-infectious skin abnormalities. Whether any of these conditions predispose to skin cancer is doubtful, but they constitute one more uncontrolled variable in an already imposing array. In any case, the question comes whether this low incidence might be construed as evidence against, rather than for the carcinogenicity of inorganic arsenites.

Massive arsenite dosage is still used in the treatment of leukemia. The heroic nature of such therapy disregards early signs of arsenic poisoning (Goodman and Gilman, *loc. cit.*). Arsenite intake in the 16 patients for whom Neubauer obtained records averaged 28 g. arsenic trioxide, thousands of times as much arsenic as that ingested normally in food.

Dr. J. A. Paris, FRS, a prolific but fanciful writer, first implied carcinogenicity of arsenic for cows in 1820, long before there was any real knowledge of the causes of cancer (*Pharmacologia, Third Edition. London* (1820)). He further implied that arsenic in the fumes of copper smelting works caused not only blight of vegetation in the surrounding area and cancer of the rumps of cows, but cancer of the scrotum in the smelters, similar to that seen in chimney sweeps. Henry Butlin (*Brit. Med. J.* 2, 66 (1892)), as part of a magnificent study of the epidemiol-



ogy of cancer of the scrotum, visited the areas described 75 years earlier by Dr. Paris. Butlin eliminated arsenic as a likely cause of cancer of the scrotum on logical grounds, but suggested instead that repeated exposure of workmen to soot, paraffin or tar, without good personal hygiene, must lead to this unique form of cancer. This careful search helped point the direction decades later to discovery of the true carcinogenic hydrocarbons in these materials.

Both Kennaway and Neubauer entreated cancer researchers to desist from citing Paris' writings as serious evidence for carcinogenicity of arsenic. But still the practice persists. Kennaway likened this phenomenon to "the strange viability of the false."

The remarkable discovery now, 140 years later, is that Paris' description of toxicity in horses and cattle does not fit arsenic toxicity at all, but rather is an apt description of selenium toxicity, viz., "Horses and cows commonly lose their hoofs, and the latter are often to be seen crawling on their knees" (see A. L. Moxon, *South Dakota Agricultural Experiment Station Bulletin No. 311* (1937)). Copper ore is now an important source of selenium. Copper smelting areas are apt to be blighted much as Paris described, but chiefly from sulfur dioxide. Repeated exposure to either arsenic or selenium dust may cause redness and burning of the skin, but exposure among smelters is now carefully avoided. Such exposure might well account for Paris' citation of "a gangrenous appearance of these parts." Now, however, after long industrial experience, there is no evidence that dermatitis of this type ever results in skin cancer. Paris probably never heard of selenium but knew arsenic as a toxin.

Dr. V. R. Potter, Professor of Oncology at Wisconsin, suggested that Paris' description appeared to fit the symptoms of selenium toxicity in cattle. This was confirmed to the author by Drs. A. L. Moxon

and O. E. Olson. All three are well acquainted with selenium toxicity in farm animals. Thus it would appear that this ancient myth, which has played a prominent part in cancer literature for 140 years, should finally be laid to rest.

Cirrhosis with adenomas or low grade carcinomas were reported in rats held 18 to 24 months on diets containing 5 to 10 p.p.m. selenium, either as inorganic selenides or seleniferous grains (A. A. Nelson, O. G. Fitzhugh, and H. O. Calvery, *Cancer Research* **3**, 230 (1943)). Thus selenium is now categorized as a carcinogen. But selenium recently acquired essential nutrient status in rats and chicks through the classic isolation research of K. Schwarz and C. M. Foltz (*J. Am. Chem. Soc.* **59**, 3292 (1957)) and of E. L. Patterson, L. Milstrey and E. L. R. Stokstad (*Proc. Soc. Exp. Biol. Med.* **95**, 617 (1957)). Prevention of exudative diathesis in chicks by selenium was reported by the latter group and by Schwarz *et al.* (*Proc. Soc. Exp. Biol. Med.* **95**, 621 (1957)). Selenium was then found to prevent white muscle disease in sheep on forage deficient in this element (O. H. Muth *et al.*, *Science* **128**, 1090 (1957)).

A. L. Moxon (*South Dakota Agricultural Experiment Station Bulletin No. 311* (1937)) and A. F. Trelease and O. A. Beath (*Selenium. Champlain Printers* (1949)) reviewed the agricultural problem posed by the high selenium content in soils of at least 14 of our western states. Moxon (*Science* **88**, 81 (1938); *Trace Elements*, p. 175 (1958)) discovered that inorganic arsenic salts counteract experimental selenium toxicity in rats. Similar value for arsonic acid in feeds was reported in rats (C. Hendrick, H. L. Klug and O. E. Olson, *J. Nutrition* **51**, 131 (1953)); in chickens (C. W. Carlson *et al.*, *Poultry Sci.* **33**, 768 (1954)); in pigs (R. C. Wahlstrom, L. D. Kamstra and Olson, *South Dakota Agric. Exp. Sta. Bull. No. 456* (1956)); and tentatively in cattle (J. A. Minyard, C. A. Dinkel and Olson, *J. Animal Sci.* **19**, 260 (1960)). Thus far,



greatest application of this principle has come in the use of arsonic acids in rations for swine (Wahlstrom and Olson, *J. Animal Sci.* **18**, 141, 578 (1959)). Counteraction of selenium toxicity by arsenicals appears to deserve research support in agriculture.

The interesting dichotomy for selenium goes still farther in that it, like arsenic, has been studied at various times as a cure for cancer (Moxon and M. Rhian, *Physiol. Rev.* **23**, 305 (1943)). In any case, the chances seem just as great that a relatively high dietary level of any such element will reduce or deter abnormalities as that it will induce them.

The idea that nature alone provides the only proper food for man is both short-

sighted and incorrect. Nature is not beneficent in creating soils in some areas of the world so high in selenium that plants growing there prove toxic to animals which consume them. By the same token, however, some soils produce forage too low in selenium to protect sheep from an apparent deficiency of this element. Natural occurrence in foods of nutrients which may also be reported carcinogenic poses problems which can be readily handled using judgment and common sense. Suitable tolerances based on knowledge can be worked out.

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## HUMAN GROWTH HORMONE EFFECTS

*The effect of human growth hormone on nitrogen retention and other metabolic balances was compared with pretreatment control values determined in a pituitary dwarf.*

Treatment of human cases of pituitary dwarfism has been difficult for a number of reasons. Preparations of growth hormone from cattle are plentiful but ineffective in the treatment of human pituitary deficiency states. C. H. Li and H. Papkoff (*Science* **124**, 1293 (1956)) compared amino acid patterns and physical constants of human and beef growth hormone preparations. They believed that the physical differences might account for the inability of beef growth hormone to substitute for the human material.

J. C. Beck, E. E. McGarry, I. Dyrenfurth and E. H. Venning (*Science* **125**, 884 (1957)) compared the metabolic action of growth hormones prepared from monkey and from human pituitaries. The hypopituitary patient responded to either preparation with nitrogen and electrolyte, as well as weight gain, indicating a similarity between these two growth hormones. The fact that only the human hormone reduced glucose tolerance and the monkey hormone caused personality modification and anuria sug-

gested the presence of impurities or inherent differences in the action of these two substances.

Other studies of the action of human growth hormone have been reviewed recently (*Nutrition Reviews* **16**, 253 (1958)). In this previously reviewed report, retention of nitrogen, phosphorus and potassium occurred in two male subjects with no pituitary insufficiency, and there was a small retention of sodium and chloride. When fecal calcium was considered in addition to urinary calcium, one subject showed a deficit and the other a positive balance for this ion. Furthermore, injection of purified human growth hormone into three hypophysectomized diabetic patients receiving insulin had a marked diabetogenic effect in that hyperglycemia, glycosuria and ketonuria resulted.

The number of studies using human growth hormone are comparatively few and accurate determination of the true metabolic effects of the pure hormone are highly desirable. J. J. Hutchings, R. F.

Escamilla, W. C. Deamer and C. H. Li (*J. Clin. Endocrinol. and Metab.* **19**, 759 (1959)) administered a highly purified human growth hormone preparation to a patient with pituitary dwarfism. By zone electrophoresis, ultracentrifugation and amino acid analyses, this substance was found to be homogenous. Its growth promoting ability, tested in hypophysectomized rats, was comparable to that of the bovine hormone. Conventional assays for thyrotropin, prolactin, adrenocorticotropin and gonadotropins showed freedom from these hormones.

The patient, an eleven and one half-year-old girl, had been seen earlier at the age of eight and three quarters years. The mother was five feet one inch in height and the father five feet seven inches. At six months the infant had a respiratory infection and high fever persisting for several weeks. After this, there was some slowing of growth and physical development. The diagnosis was pituitary dwarfism secondary to encephalitis.

At the time of the first hospital admission at eight and three quarters years, tests revealed a low thyroidal  $I^{131}$  uptake and an adequate response to thyrotropin. For this reason sodium L-thyroxine was given for two and a half years in an effort to improve the growth rate. No stimulation of growth resulted, but bone maturation improved. Six weeks before the present study treatment was stopped.

The administration of growth hormone was alternated with control periods without medication. After an initial control period, 5 mg. of growth hormone were given daily for ten days followed by a one-week control period. Then a larger dose (10 mg. of hormone) was given daily for five days, again followed by a week without medication, after which the 10 mg. dose was again administered daily for five days. Following a final control period of five days, 2.5 mg. of hormone were given daily for ten days.

The daily diet intake was fixed at 1600 calories, 172 g. of carbohydrate, 70 g. of protein, and 70 g. of fat. The intake of sodium, potassium, calcium and phosphorus was also held constant. All urine and stools were collected and analyzed.

During each period of hormone administration the patient gained weight, but during each control period she lost weight. A striking decrease in urinary nitrogen excretion was observed during each period of therapy with human growth hormone. This type of immediate nitrogen retention does not occur in human subjects when bovine growth hormone is used. Accompanying this nitrogen retention was a decrease in blood urea nitrogen followed by a return to control levels when the drug was stopped. Although retention of nitrogen occurred at all dosage levels, the 5 mg. dose appeared to be optimal.

Two methods have been described for the preparation of growth hormone, the first by M. S. Raben (*Science* **125**, 883 (1957)) and the second by Li and Papkoff (*Ibid.* **124**, 1293 (1956)). Both preparations have been used successfully in promoting nitrogen retention in cases of pituitary insufficiency. The preparation of Li and Papkoff was also able to promote anabolism in normal subjects (*Nutrition Reviews* **16**, 253 (1958)).

In the study under discussion, sodium, potassium and phosphorus were retained during the experimental periods. Serum alkaline phosphatase rose slightly, but the serum levels of inorganic phosphorus and other ions did not change. Calcium balance was negative in the short experimental periods, but when 2.5 mg. of human growth hormone were given daily for two months the calcium balance became positive.

Fasting blood sugar levels were not altered by the administration of growth hormone and no glycosuria was discovered. Glucose tolerance curves, however, were higher in both acute and long-term trials.

Significant changes did not occur in the level of serum protein-bound iodine, or in

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urinary 17-ketosteroids, 17-hydroxy-corticoids or gonadotropin.

The administration of human growth

hormone produced a growth of 9 cm. over a period of nine months in contrast to a growth of 3 cm. per year before treatment.

### FINGERNAIL GROWTH IN HEALTH AND DISEASE

*Marked individual variation occurs in the growth rate of fingernails in human subjects. Certain disease states have profound effects on nail growth while malnutrition and dietary restrictions do not.*

Previous studies on nail growth (*Nutrition Reviews* 15, 327 (1957)) have demonstrated that photographic techniques give reproducible results on nail growth even over short periods of time. The rate of nail growth in males increases from 0.10 mm. per day in infants to 0.11 mm. in adolescents and then gradually decreases to 0.08 mm. at the age of 70. Nail growth in females may be slightly less. Evaluation of nail growth as a measure of nutritional status is incomplete but it has been noted that such things as chilling, illness or immobilization of the digit will reduce nail growth.

A study of growth of fingernails in infants and children by M. S. Sibinga (*Pediatrics* 24, 225 (1959)) using a photographic technique with 35-fold enlargement has permitted day to day measurement. Variations in nail growth between normal adults were large, varying between 1.9 and 4.4 mm. per month. Consequently each individual must serve as his own control. In two groups of healthy young adults comprising 81 individuals of both sexes 25 per cent variation was found, about a mean of 0.106 mm. per day. No consistent sex differences were found in rate of growth, although there were transverse ridges 2 to 4 mm. apart in the females which might correspond to monthly growth arrests. However, these were not specifically related to menstruation because the studies were not carried out over a long enough period of time. Such lines were not observed among the males.

Observations on nail growth in ten normal infants under six months of age showed

average daily growth within the range found in adults. Some of these infants were subjects of nutritional studies carried out by S. E. Snyderman, A. Boyer and L. E. Holt, Jr., (*A.M.A. J. Dis. Child.* 97, 192 (1959)) in which single amino acids were omitted from the diet over short periods of time. They showed no change in nail growth despite the fact that gain in weight was temporarily arrested. It would appear that nail growth was not a particularly sensitive criterion of this type of experimental nutritional deficiency.

B. Schick (*Jahrb. Kinderh.* 67, 146 (1908)) observed transverse ridging of the nail laid down at the time of birth. This ridging was recognized in only three of 14 infants studied during the first week of life. The nail growth of 13 premature infants varying in weight from 1150 to 1900 g. was measured over a period varying from 13 to 15 days. Their nail growth was found to be in the same general range as that of full-term infants and adults, although the average was somewhat lower. The findings did not correlate with either birth weight or weight gain.

Observations made by Sibinga (*loc. cit.*) on 23 patients with measles and high fever showed complete arrest of nail growth for periods of variable duration. Ten children with febrile tuberculosis had rates of nail growth that were within normal range. Single instances of mumps and staphylococcal septicemia produced a cessation of nail growth for approximately a week. The nail growth of seven obese adults on a 600-calorie reducing diet for prolonged

periods showed growth rates that fell within the lower half of the normal range.

The daily growth of nails was observed post mortem in three adults for periods of eight to ten days' duration. For the first two or three days after death nail growth

was in the low normal range, while growth continued at a somewhat slower rate during the remainder of the observation period. The author notes with interest that measles had a greater depressing effect on nail growth than death.

## PYRUVATE METABOLISM IN WILSON'S DISEASE

*The abnormal pyruvate levels in the blood of patients with Wilson's disease can be reduced by giving sulfhydryl compounds, which may be needed for intermediary metabolism in the cells.*

The role of increased copper deposition in the tissues of patients with Wilson's disease (hepato-lenticular degeneration) has been discussed earlier (*Nutrition Reviews* **12**, 198, 304 (1954); **13**, 232 (1955); **16**, 37 (1958)). In the therapy of this disorder agents are used which free copper from the tissues and cause it to be excreted in the urine (cupuresis). However, a poor correlation has been noted between the observed clinical response and the degree of cupuresis. The substances commonly used to remove copper from these patients are dimercaprol (BAL) and D-mercaptovaline (penicillamine), both containing sulfhydryl groups. It is believed that the sulfhydryl groups bind the copper in a soluble form, thus allowing it to be excreted by the kidney.

J. M. Walshe (*Am. J. Med.* **21**, 487 (1956)) has suggested that perhaps the copper in tissue might block the naturally occurring sulfhydryl groups present in enzyme systems of the Krebs citric acid cycle. If this were so, however, one might expect an interference of the entry of pyruvate into the Krebs cycle and a resultant accumulation of keto acids in the blood.

Recently L. Hill and J. M. Walshe (*Lancet* **II**, 444 (1959)) made a preliminary report on the pyruvate levels in the blood of three patients with Wilson's disease. The first individual was a 20-year-old man who had been given penicillamine for two years and,

before that, dimercaprol for several months. The second patient was a 21-year-old man who had received no previous therapy, and the third patient was a 13-year-old girl who had received penicillamine for four months before beginning the study. As control subjects, these investigators chose two male patients (62 and 46 years old) who had cirrhosis of the liver and elevated pyruvate levels following glucose administration.

In all the studies, changes in the pyruvate levels were measured before and after loading with glucose. Each patient was given 50 g. of glucose at zero time and again 30 minutes later, with blood samples being obtained at 0, 60 and 90 minutes. The levels of both pyruvate and lactate were determined in the blood samples. To negate any effect of a previous vitamin deficiency, the patients were given 100 mg. of thiamine before the study was started.

In the first patient, following the glucose load and before treatment for removal of copper, the pyruvate and lactate levels were at the upper limits of normal and, therefore, this patient was not studied further. The authors believe that these high levels were caused by earlier treatment for a very long period with agents to remove excessive copper deposits which freed the bound sulfhydryl groups necessary for the metabolism of the keto acids.

The second patient had high pyruvate and lactate levels in the blood before treat-

ment, both in the fasting state and after receiving the glucose load. However, after receiving penicillamine (900 mg. per day) for three days the pyruvate levels returned to the upper limits of normal.

The third patient had a grossly abnormal pyruvate tolerance test before treatment, but after receiving 900 mg. of penicillamine per day for three days the pyruvate levels after glucose feedings fell within the upper limits of normal. After therapy was stopped, it took two weeks for the pyruvate values to attain the high pretreatment level. When a second course of penicillamine was given, the blood pyruvate levels again returned to normal. Later, this patient received a course of treatment with N-acetyl penicillamine and changes in the blood pyruvate concentrations following the glucose load were similar to that produced by penicillamine. This was of interest since the N-acetyl penicillamine does not induce increased excretion of copper in the urine.

The control patients who had hepatic cirrhosis also had abnormal pyruvate tolerances, but these were not influenced by treatment with penicillamine.

These investigators believe that the beneficial effect of the sulfhydryl chelating agents in Wilson's disease may be in part due to their supplying or unmasking sulfhydryl groups needed for the intermediary metabolism of the cells. It should be noted that, while N-acetyl penicillamine does not remove copper as indicated by a cuperesis, it does cause an improvement in pyruvate tolerance, and this may be due to its sulfhydryl groups. While this problem is not entirely solved, the investigators are studying the effects of other chelating agents which do not have sulfhydryl groups.

Increased levels of pyruvate and lactate in the blood following the administration of a glucose load were noted by D. Henneman and J. P. Bunker (*Am. J. Med.* **23**, 34 (1957)) in patients with Cushing's syndrome (increased adrenal cortical activity) or in patients given 17-hydroxycorticosteroids. These investigators believed that there was a direct inhibition of the conversions of pyruvate to carbon dioxide and acetyl coenzyme A by the steroid rather than an effect on the tricarboxylic acid cycle.

### VITAMIN B<sub>6</sub> IN HUMAN MILK

*Human milk, which may be poor in vitamin B<sub>6</sub>, can be effectively enriched by oral administration of pyridoxine.*

Because of the almost ubiquitous presence of vitamin B<sub>6</sub> in human dietary constituents, a spontaneous B<sub>6</sub> deficiency in the human adult occurs very infrequently if at all (*Present Knowledge in Nutrition, The Nutrition Foundation, Inc., New York (1956)*). However, some cases of infant deficiency of this vitamin have been studied. For example, one proprietary formula for infants was so low in vitamin B<sub>6</sub> that many of the babies using it developed symptoms of the deficiency such as hyperirritability, convulsions and, in some cases, xanthurenic acid excretion (*Nutrition Reviews* **16**, 10 (1958)). Fortification of the formula with

200 micrograms of pyridoxine per liter or removal of the protein prevented the development of the symptoms. From these and other studies, it was found that about 300 micrograms per day prevented deficiency symptoms in most normal infants.

According to R. Karlin (*Bull. Soc. Chim. Biol.* **41**, 1085 (1959)), a borderline deficiency may very well exist in infants since human milk is relatively poor in vitamin B<sub>6</sub>. During the first few days post partem human milk may contain as little as 1 to 2 micrograms per 100 ml., while the mean for 85 normal women from one to seven months postpartem was 10.5 micrograms per 100

ml. as compared with 76 for cows. It is possible that this low value in the milk of nursing mothers reflects some special drain on their resources during pregnancy, indicated by the excretion of large amounts of xanthurenic acid during pregnancy (M. Wachstein and A. Gudaitis, *J. Lab. Clin. Med.* **40**, 550 (1952)). In any event, Karlin (*loc. cit.*) has investigated the most efficient method of raising the milk of nursing mothers to an acceptable level.

Three methods of administration of the vitamins were studied. The first consisted of an intramuscular injection of 50 mg. of pyridoxine, after which the level in the milk was ascertained at various intervals for eight days. For the second method, four tablets containing 250 mg. of pyridoxine were taken orally with a glass of water and the same analyses were conducted as for the injection method. For the third method two or four tablets were taken each day for five days and analyses were performed both during the treatment and for six days afterward. In each instance, the vitamin was administered in the post-

absorptive state, and samples for analysis were taken under the same conditions where possible.

Following the injection, the level of vitamin B<sub>6</sub> in the milk increased from 13.5 micrograms per 100 ml. to a maximum of 130 at three hours, followed by a precipitous drop to 43 at nine hours and a more gradual fall to 20 micrograms per 100 ml. at 96 hours. Following the single oral dose, milk levels reached a maximum of about 1000 micrograms at three hours and then fell rather slowly to about 20 micrograms at seven days. During the daily oral doses the milk level of vitamin B<sub>6</sub> remained high (100 to 400 micrograms per 100 ml.) and then fell slowly to about 20 micrograms six days after the last dose. In the oral administration studies, 0.5 g. doses appeared to be as effective as 1 g. doses.

The author has thus demonstrated the feasibility of enriching the milk of nursing mothers by oral administration of pyridoxine. Thus prevention of a possible deficiency in infants is both possible and practicable.

## PLASMA LIPID FATTY ACIDS

*Gas-liquid chromatographic analyses of fatty acids from various plasma lipid fractions of normal fasting subjects were compared with the same analyses done on plasmas taken after eating.*

The blood plasma of a 70 kg. man contains about 50 mEq of fatty acids at any one time. During the transport of about 300 mEq of fatty acids from a single meal containing 3 ounces of fat it would be reasonable to expect a similarity in the fatty acid pattern of diet lipid and plasma lipid.

W. R. Wilson and J. P. Hanner (*J. Biol. Chem.* **106**, 323 (1934)) observed a change in the iodine number of blood lipids after eating, corresponding to the iodine number of cream or cod-liver oil fed to the young subjects. J. Hirsch, J. W. Farquhar, M. L.

Peterson and W. Stoffel (*J. Clin. Invest.* **38**, 1011 (1959)) observed that feeding large amounts of unsaturated fatty acids to man altered the composition of depot lipids. This, too, suggested that much of the diet fat was transferred unaltered. J. Fernandes, J. H. Van de Kamer and H. A. Weijers (*J. Clin. Invest.* **34**, 1026 (1955)) studied a child with chylothorax. They found that, while significant differences between chyle and diet fat remained, the fatty acid pattern of chyle was definitely influenced by the kind of fat fed during the preceding week. Long-term feeding of a particular fat with a

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distinctive fatty acid pattern eventually results in alteration of plasma lipid fatty acids.

In order to learn whether plasma lipid fatty acids sampled after a meal resemble dietary lipid, V. P. Dole *et al.* (*J. Clin. Invest.* **38**, 1544 (1959)) studied fatty acid patterns of plasma lipids in fasting human subjects as compared to subjects fed corn oil, coconut oil or butter.

Four main fractions of plasma lipid fatty acids were separated, methyl esters formed and analyses completed using gas liquid chromatography. When the early type of gas density balance was used as the detector, 3 mg. of methyl esters were required necessitating 50 to 75 ml. of plasma. Later, analyses using the more sensitive argon ionization detector reduced the methyl ester sample requirement to 100 micrograms and the plasma sample to 10 ml. The fractions, which were isolated and analyzed, included chylomicra, non-esterified fatty acids, phospholipids and a mixture of triglycerides and cholesterol esters.

For control data, samples of plasma were taken from human subjects 12 hours after eating and from fasting animals maintained on commercial feeds. For the feeding experiment, fasting subjects were bled then fed 100 g. of test fat (corn oil, coconut oil or butter) with no other food. For additional observations, three blood samples were taken at one half, one, and two and one half hours in the short-term studies, or at three, six and nine hours in the longer term studies.

Symbols were used to identify the numerous fatty acids present in animal fat. The chain length of the fatty acid was written first, then the number of double bonds. For example, 18:0 represented stearic acid, 18:1 oleic acid, and 18:2 linoleic acid. Other information was stated, such as "branched 18:0", while the location of double bonds was indicated as 18:1<sup>9-10</sup>. In spite of this simplification, recording of the

analyses was complex and it was difficult to compare mixtures of fatty acids differing in minor components.

In order to summarize degree of saturation in any specific mixture of fatty acids, the double bond index was determined. This number was the sum of calculations made for each individual fatty acid as follows: the product of the number of double bonds times the per cent by weight of fatty acid divided by the molecular weight of that fatty acid.

Comparison of the composition of the various diet lipids with plasma lipid fatty acid patterns in the short-term experiment showed no change in pattern before and after feeding with one exception; in the case of coconut oil there was a rise in lauric and myristic acids after the test meal.

In longer term experiments, the changes were significant but of minor degree. When corn oil was analyzed, 34 per cent was found to be oleic acid and its isomers and 55 per cent linoleic acid. Nevertheless, after feeding corn oil, the proportion of linoleic acid in each plasma fraction showed only moderate elevation while the oleic acid remained unchanged or decreased. Under these conditions, chylomicra showed a perceptible effect from the dietary load even though the predominant tendency was to maintain the pattern found in the fasting samples. On the assumption that the plasma lipid pattern during absorption of diet fat was composed of a mixture of dietary fat and body lipids with the fasting pattern, it was concluded that about half the oleic acid of chylomicra came from the diet while a much smaller proportion of the other components were influenced by the diet load.

The nonesterified fatty acids were also noticeably influenced by the diet, but the fatty acid patterns of phospholipids and of triglyceride plus cholesterol esters were unchanged.

In a previous study, A. T. James, J. E. Lovelock, J. Webb and W. R. Trotter



(*Lancet* I, 705 (1957)) observed that each class of blood lipid had a distinctive fatty acid composition with relatively small variation in concentration of either major or minor components. This observation was verified in the present study.

Polyunsaturated acids were highly concentrated in chylomicra. This could have been due to the presence of highly unsaturated phospholipid or triglyceride components. At any rate, there must have been an endogenous source rich in polyunsaturated acids.

The data in the present study may be compared with data in the study with British subjects by James *et al.* (*loc. cit.*). The phospholipid composition was similar, except that palmitic acid was higher in the American group while arachidonic acid was lower. In the cholesterol ester plus triglyceride fraction, the American group had 33.5 per cent linoleic acid and the British 12.4 per cent. The British oleic acid level was 34.7 and the American 26.4 per cent. These differences may have been due to a higher cholesterol-triglyceride ratio in the American subjects.

Dole *et al.* also examined the fatty acid patterns in the serum lipid fractions of the sheep, cow, horse, pig and chicken and found a surprising similarity despite the wide differences in foods eaten by the various species.

I. Bang (*Biochem. Z.* **91**, 111 (1918)) observed that the amount of postprandial lipemia depended on the kind of fat and the amount fed. A Hiller *et al.* (*J. Exp. Med.* **39**, 931 (1924)) noted that moderate loads of fat, such as might be eaten in the usual

meal, cause little rise in the lipid concentration of plasma. Feeding 3.5 g. per kg. causes a substantial rise in plasma fatty acid concentration, however.

In the present experiment, the moderate feeding of corn oil produced a slight rise in plasma triglyceride. When coconut oil was given, no significant change was seen. All subjects showed turbidity of plasma during absorption, reaching a peak at six hours. Centrifuging the turbid plasma with saline layered on top yielded a layer of creamy chylomicra, the composition of which differed from diet fat.

Several factors might influence the nature of chylomicron fatty acid pattern. The presence of phospholipid and cholesterol ester components would furnish fatty acids not related to the immediate dietary spectrum. Furthermore, the presence of contaminating non-chylomicron lipids would influence the pattern of these lipids. Nevertheless, these particular factors would not be enough to account for the large difference between the fatty acid composition of chylomicra and dietary fat. Phospholipids and cholesterol esters furnish only about 10 per cent of the fatty acids in chylomicra.

The authors offered two possible explanations for the stability of the fatty acid patterns of plasma lipids during absorption. One, also suggested by W. R. Bloor (*J. Biol. Chem.* **16**, 517 (1913-14)), was the conversion of fatty acids during digestion. The other proposal was that fatty acids might be rapidly recycled between blood and tissue pools. Experiments by these authors, currently in progress, suggest that the latter mechanism is correct.

## TRACE ELEMENTS AND DENTAL CARIES

*The influence of several trace elements on the caries-susceptibility of rodent teeth has been tested by injection into suckling rats.*

Various trace elements other than fluorine have been studied to determine their influence on susceptibility to experimental dental

caries. Most studies have been concerned with the period after the teeth are calcified, although a few have been conducted during

tooth development. In one early study, supplements of zinc, barium and thallium were said to cause poor calcification of the long bones and a high incidence of tooth decay, while supplements of strontium and vanadium promoted good calcification and a reduced incidence of tooth decay (*Nutrition Reviews* 8, 178 (1950)). These data must be viewed with skepticism and the same minerals retested under satisfactory experimental conditions.

The whole area of mineral supplements to cariogenic diets has been reviewed by J. W. Hein (*Advances in Experimental Caries Research*, p. 197. Edited by R. F. Sognnaes. American Association for the Advancement of Science, Washington, D. C. (1955)). He pointed out the wide areas of confusion in this field; the variations in response between different species of experimental subjects and between results from different laboratories, as well as the lack of correlation between *in vitro* trials and *in vivo* investigations.

The influence of trace elements on tooth development is difficult to evaluate in rodents because of the advanced state of calcification and eruption at weaning. In most studies the trace elements have been added to the diet or the drinking water of the mother, with the realization that the actual amount received by the fetus or the suckling was unknown and might be reduced to an insignificant level in the passage across the placenta and the mammary gland.

In order to guarantee that the desired dosage was made available to the suckling rat, J. Kruger (*Univ. of Queensland Papers, Dept. of Dentistry* 1, 1 (1959)) has conducted a series of experiments in which various trace elements were injected intraperitoneally between the fifth and seventeenth days of age. In the first experiment, 0.15 mg. of manganese as manganese sulfate in saline solution was injected daily into 14 rats. Fifteen unsupplemented controls were kept from the same litters. No significant difference be-

tween the rats in the two groups was observed, although a tendency toward an increased caries incidence among the manganese-supplemented rats was noted.

In the second experiment, a solution containing five minerals was injected into 16 experimental rats. Each rat received a daily supplement of 0.005 mg. of copper as copper nitrate, 0.005 mg. of boron as boric acid, 0.002 mg. of molybdenum as ammonium molybdate, 0.05 mg. of fluorine as sodium fluoride, and 0.05 mg. of manganese as manganese chloride. The result of this experiment was not definitive, but probably there was no important reduction in caries incidence. Although a significant reduction at the 5 per cent level was observed in the number of grossly visible carious lesions in the experimental rats, the two other criteria used, the total dental caries incidence and the caries score, did not differ significantly between groups.

In the third experiment, the same five elements were tested at the same levels. The fluoride supplement was tested alone in one group, the other four supplements were tested together in another group, and all five supplements were tested together in a third group. A fourth group of controls received only the saline solution. The fluoride supplement caused a highly significant reduction in dental caries incidence, but the solution with the other four minerals did not cause a significant reduction. When all five minerals were given in a single injection, there was a highly significant reduction, which, however, was not as great as when the fluoride supplement was given alone. It is interesting that the supplement of five minerals in this experiment caused a highly significant reduction while it had been relatively ineffective in the previous experiment.

Unfortunately, beginning with this experiment and throughout the rest of the paper, no raw data are presented and the case is allowed to rest on the tabulation of statistical analyses. This deficiency makes it impossible for the reader to make a good eval-

uation of the strain and its suitability for this type of experiment.

In the fourth experiment, 60 rats from ten litters were distributed in a complicated design to test the influence of these five minerals singly and in pairs at two dosage levels, that used in experiments two and three, and a higher level varying from two to five times the lower level. Each rat received a different treatment combination. The results are difficult to evaluate because the significance of the reductions varies depending upon the criterion for evaluation, *i.e.*, the number of gross carious lesions, the total number of detectable lesions, or the caries score. The higher dose of boron caused a significant reduction at the 5 per cent level as judged by all three criteria. The higher dose of molybdenum caused a significant reduction at the 1 per cent level for the number of gross lesions, but the reductions for the other two criteria were only significant at the 5 per cent level.

Interestingly enough, fluorine at the same level used in experiments two and three was ineffective when judged by the number of gross lesions and the total number of detectable lesions, but the reduction in caries score was significant at the 5 per cent level. This variability in results suggests that the complicated design may have been unduly sophisticated for the population of rats under investigation.

In a fifth experiment, another complicated design with 16 litters of four rats each was used to test the influence of daily supplements of 0.054 or 0.108 mg. of fluorine as sodium fluoride, 0.005 or 0.025 mg. of boron as boric acid, 0.002 or 0.005 mg. of iodine as presumably free iodine, 0.008 or 0.025 mg. of aluminum as aluminum acetate and 0.005 or 0.025 mg. of vanadium as vanadium chloride. The rats were given all five elements in different combinations of low or high doses, and only one rat received a particular combination.

Again the results are difficult to interpret because of the differences in significance be-

tween the number of gross lesions and the number of detectable lesions. By both criteria of evaluation, the increased fluoride level and the increased boron level caused significant reductions in dental caries. Iodine, aluminum and vanadium were ineffective on the basis of the number of gross carious lesions, but vanadium appeared to be moderately effective on the basis of the number of detectable lesions. There appeared to be a mild interaction between fluoride and boron when judged by the reduction in the number of gross lesions, but no influence on the total number of detectable lesions was noted. On the other hand, there appeared to be a highly significant interaction between fluoride and aluminum and between aluminum and vanadium when judged by the total number of detectable lesions, although no interaction had been observed for the number of gross lesions.

In the sixth experiment, four different levels of copper (from zero to 60 micrograms) and of boron (from zero to 19.5 micrograms) were tested in a replication experiment involving 12 litters of four rats each, with a different level of boron and copper for each rat. The results were completely negative for both boron and copper. Although the highest boron level was slightly lower than the lower level used in other experiments, there was no suggestion of a trend toward caries reduction. The copper level was higher than in the previous experiments but did not give any further information on the trend reported toward a significant reduction.

The author attributed the widely fluctuating caries values observed in this experiment to "some experimental error." Judging from the breadth of data on experimental caries available from other laboratories, the experimental error probably lay in the use of a design which was too complicated and which employed too few subjects for the widely variable population of rats being used as the source of subjects.

These experiments represent sincere efforts to appraise the role of several trace

elements in the etiology of dental caries by daily intraperitoneal injections of the test doses into suckling rats from the fifth to the seventeenth day of age. However, the author has succumbed to excessive dependence upon statistical designs in the effort to test numerous variables simultaneously. Unfortunately, the nature of investigations on dental caries and the variability of the average rat popu-

lation with respect to caries-susceptibility presently preclude the use of such complicated designs. A similar amount of investigation using several simple variations could have produced results in which more confidence could be placed. As it is, only fluoride and boron appear to be effective, and even with these some of the experiments are not conclusive.

### BODY WEIGHT AND ENZYMES

*The activities of a number of enzymes in the bodies of a variety of animal species are related to the body weight<sup>0.75</sup>. This relationship is similar to that for the basal metabolic rate.*

Ever since M. Rubner suggested that the basal metabolic rate was related to the mass of "active tissue", a number of attempts have been made to explain the relationships observed (A. Keys *et al.*, *Biology of Human Starvation*, vol. 1, p. 312. Univ. of Minnesota Press (1951)). Rubner found a fairly constant relationship between the hypothetical "active cell surface area" and basal heat production. When he applied the calculations developed for man to the mouse, the energy expenditure was 11 times as great per square meter of active surface as in man. It is interesting that more recent studies suggest that the concentration of certain oxidative enzymes in the kidneys of mice is ten times that in humans (Keys *et al.*, *loc. cit.*).

A number of years ago, D. L. Drabkin (*J. Biol. Chem.* **182**, 317 (1950)) started to explore the relationship between body size and the concentration of such chromoproteins as hemoglobin, myoglobin and cytochrome *c* in the tissues of a number of animal species. For the rat, dog, heifer, horse and man, the amount of hemoglobin was directly proportional to body mass, with 12.7 g. of hemoglobin in 1 kg. of body weight. In all of these species except the horse, the total amount of cytochrome *c* was proportional to the body weight<sup>0.7</sup>. There was 3.0 millimole of cytochrome *c* per kilogram of body weight<sup>0.7</sup> for the above four species, while for the horse,

the figure was more than five times this. When the cytochrome *c* content in the organs of man and the rat was determined, it was found that 80 per cent of the enzyme was present in skeletal muscle, and when heart, liver and kidney were also included, the value was 93 per cent.

Drabkin suggested that, in spite of the similarity between the relationships of cytochrome *c* content and basal oxygen consumption to the body weight<sup>0.7</sup>, the cytochrome *c* content probably was related more closely to "metabolic capacity" rather than "basal metabolism." Data collected and analyzed by Drabkin indicated that in the resting or basal state, the oxygen consumption of the brain, heart, kidney and liver accounted for 70 per cent of the total while the muscles accounted for only 13 to 16 per cent. Since the muscles contained 80 per cent of the cytochrome *c*, the concentration of this enzyme in muscle was obviously not related to the basal metabolic rate of that tissue.

The above work was extended by H. O. Kunkel, J. F. Spalding, G. de Franciscis and M. F. Futrell (*Am. J. Physiol.* **186**, 203 (1956)). They found that the cytochrome oxidase activity per unit weight of gracilis muscle in adult male rats and female swine was related to the logarithm of the body weight by a correlation coefficient of  $-0.97$ . The corresponding correlation coefficient for

the ruminants used in the study (adult male and castrated male beef cattle and adult castrated male and female sheep) was  $-0.81$ ; for all species (numbers of animals not stated) the correlation coefficient was  $-0.89$ .

It was found that the activity of the cytochrome oxidase in each gram of liver was not related to the body weights of the rat, sheep, swine or cattle. Furthermore, the weights of the livers were not related to the body weights of the animals. However, the logarithms of the total liver cytochrome oxidase activity and the logarithms of body weights were closely related, as shown by a correlation coefficient of  $-0.98$ . Calculations indicated that the cytochrome oxidase activity was related to the body weight $^{-0.75}$ . On this basis, Kunkel *et al.* concluded "that the summated total measurable cytochrome oxidase activity is very nearly proportional to body weight to the  $\frac{3}{4}$  power and hence, to basal metabolism."

The observation that the amounts of cytochrome *c* and cytochrome oxidase were related to the body weights $^{-0.75}$  for a variety of species led E. C. C. Lin, R. S. Rivlin and W. E. Knox (*Am. J. Physiol.* **196**, 303 (1959)) to investigate whether the same were true for a group of oxidative and non-oxidative enzymes. The calculation of the amount of enzymes in the bodies of the animals was based on an earlier report by Lin and Knox (*J. Biol. Chem.* **233**, 1186 (1958)) that 90 per cent of these enzymes were in the liver.

The mice, rats, hamsters, guinea pigs and rabbits used by Lin, Rivlin and Knox (*loc. cit.*) had been maintained on commercial stock diets with carrot and lettuce supplements for the guinea pigs and rabbits. All animals were adults when they were sacrificed for the determination of the enzymatic activities of their livers.

When the logarithm of the value for the enzymatic activity of the body, calculated from the value for the liver, was plotted against the logarithm of the body weights, fairly straight lines resulted. The straight-

line relationship extended from mice with body weights of 26 g. to rabbits with body weights of 4300 g. This was true for the phenylalanine- $\alpha$ -ketoglutarate, tyrosine- $\alpha$ -ketoglutarate, and tyrosine-pyruvate transaminases, as well as for homogentisate oxidase. The values for the phenylalanine-pyruvate transaminase and the para-hydroxyphenylpyruvate oxidase activities of the guinea pigs fell below the straight line formed from the values of the other animals. It was suggested that the low para-hydroxyphenylpyruvate oxidase activity of the livers of the guinea pigs was probably associated with the excretion of large amounts of para-hydroxyphenylpyruvate when scorbutic guinea pigs are given a dose of tyrosine.

Calculations indicated that the activities of the enzymes were related to the body weights with the following exponents: phenylalanine- $\alpha$ -ketoglutarate transaminase,  $-0.54$ ; phenylalanine-pyruvate transaminase,  $-0.25$ ; tyrosine- $\alpha$ -ketoglutarate transaminase,  $-0.50$ ; tyrosine-pyruvate transaminase,  $-0.29$ ; para-hydroxyphenylpyruvate oxidase,  $-0.71$ ; and homogentisate oxidase,  $-0.73$ . The values for the activities of phenylalanine-pyruvate transaminase and para-hydroxyphenylpyruvate oxidase were excluded from the above computations since their deviations from the straight lines were more than twice the standard deviations.

Lin, Rivlin and Knox were impressed by the fact that the above values for the relationships between body weights and activities of the two oxidases ( $-0.71$  and  $-0.73$ ) were very similar to the value secured by previous investigators for the relationship of the basal metabolic rate and body weight. The values for the transaminases were much lower than this. The difference in the values for these two sets of enzymes was assumed to be related to the fact that the transaminases did not react with oxygen.

It is interesting and intriguing that the activities of a number of enzymes in the bodies of a variety of animal species show a

relationship to the body weight which approximates the value secured from basal metabolic rate data. Whether any physiological interpretation can be deduced from the similarity of the exponents for the body weights observed in these two types of data is open to question. To attempt to explain the basal metabolic rate on the basis of the enzyme activity or concentrations would appear illogical. It is very likely that the body has a considerable reserve of each enzyme, and under normal resting conditions probably only a fraction of the total enzyme

content is required for any metabolic reaction. Such a situation would appear to be necessary if the body were to be able to assume the tremendous physiological load associated with a metabolic energy such as severe physical exercise.

The work of Lin, Rivlin and Knox, however, does suggest a means of expressing the concentration of enzymes in the body which permits a comparison of animals of different weight within the same species as well as animals of different species.

## AMINO ACID IMBALANCE, II

*Amino acid imbalance may account for the increase in the lysine requirement for maximum growth with increasing dietary levels of wheat gluten, deficient in lysine.*

Many investigators have reported an increase in the requirement for the most limiting amino acid in the diet as the dietary level of protein is raised. The similarity between the condition used to study the influence of the dietary protein level on amino acid requirements and the condition used to observe amino acid imbalances, as well as observations that the requirement for the most limiting amino acid (expressed as a percentage of the diet) may be increased in both cases, suggested to S. M. Munaver and A. E. Harper (*J. Nutrition* **69**, 58 (1959)) that the two conditions have a common basis. Hence they determined to study the influence of the protein level on amino acid requirements by testing the effect of wheat gluten, deficient in lysine, on the lysine requirement of the growing rat. They also observed the influence of the dietary level of wheat gluten on the deposition of fat in the tissues and on protein digestibility.

Their report is the second in a new series of papers on amino acid balance and imbalance (Harper, *J. Nutrition* **68**, 405 (1959); *Nutrition Reviews* **18**, 113 (1960)). Harper regards amino imbalance as any change in the proportions of amino acids in the diet that results in an adverse effect

which can be prevented by supplementing the diet with a relatively small amount of the most limiting amino acid or acids (Harper, *Ann. N.Y. Acad. Sci.* **69**, 1025 (1958); *Nutrition Reviews* **14**, 225 (1956); **17**, 122, 232 (1959)).

In the initial experiments, the rate of weight gain of weanling rats fed for two weeks diets containing different levels (from 10 to 88 per cent) of wheat gluten without supplements of lysine, increased stepwise with each increase in the dietary level of wheat gluten up to 70 per cent. From 70 to 88 per cent there was no growth but neither was there a depression of growth rate. The rate of gain of rats fed different levels (from 10 to 60 per cent) of wheat gluten, supplemented in each case with 1 per cent lysine, was maximum when the diet contained 30 per cent wheat gluten and decreased as the level of wheat gluten was increased further.

Since these studies suggested that the level of lysine needed to support maximum growth increased as the level of wheat gluten was raised, diets containing 30, 47, 53 and 60 per cent wheat gluten (0.5, 0.8, 0.9 and 1.0 per cent lysine, respectively) were selected and the effects of supplementing each of these with three levels of



lysine were determined. Data from this experiment indicated that for maximum weight gain the lysine requirement increased from 0.8 to 0.9 per cent on the 30 per cent wheat gluten diet to about 1.2 per cent on the 60 per cent wheat gluten diet. Digestibility was not influenced by the dietary level of protein. Data from carcass analyses indicated that the dietary level of protein did not influence the moisture content of the rat carcasses appreciably, but did result in a slight decrease in fat content and increase in protein content as the dietary protein level was raised.

While these studies were in progress, N. Nath, Harper and C. A. Elvehjem (*Canad. J. Biochem. Physiol.* **37**, 1375 (1959)) observed that, although only traces of fat could be extracted from wheat gluten by ether, up to 12.7 per cent of lipid-like material (about 65 per cent of which was ether soluble) could be obtained by continuously extracting wheat gluten with *n*-butanol for 48 hours.

Since this lipid would cause an increase of 6.4 calories per 100 g. of diet for every 10 per cent increase in wheat gluten level, and since the lysine requirement of the growing rat increases with the caloric value of the diet (H. R. Rosenberg and R. Culick, *J. Animal. Sci.* **14**, 1221 (1955)), the preceding experiment was repeated using diets made isocaloric by the addition of corn oil with that containing 60 per cent of wheat gluten. Under these conditions, as before, the weight gain of rats fed diets containing a particular level of lysine fell off as the dietary level of protein was increased. However, the amount of lysine required to support maximal growth with the lower levels of wheat gluten was somewhat higher than observed in the preceding experiment, indicating that the effect of wheat gluten level on lysine requirement observed previously was partly a result of the higher caloric content of diets containing high levels of wheat gluten and only partly a result of the increase in the dietary level of protein.

The results of the last series of experi-

ments, in which diets containing a mixture of 30 per cent wheat gluten and 30 per cent zein were supplemented with graded levels of L-lysine monohydrochloride (0.5 and 0.8 per cent), confirm the similarity between the effect of higher levels of protein and the effect of amino acid imbalance on the lysine requirement. Replacing 30 per cent of the dextrin in the control diet with zein resulted in a significant lowering of weight gain. Additional lysine (0.3 per cent) was needed to return weight gain to control values. The prevention of growth retardation by a supplement of the most limiting amino acid is characteristic of amino acid imbalances.

From their data the authors calculated the effect of the dietary level of wheat gluten on the amount of lysine required for two weeks' maximum growth by rats consuming isocaloric diets containing about 12.5 per cent of fat. As the wheat gluten content of the diet was increased from 30 to 53 per cent, the lysine requirement for maximum gain increased from 1.0 to about 1.2 per cent of the diet, or from 1.4 to 1.6 g. of lysine for the whole two weeks.

Wheat gluten, the authors conclude, is so severely unbalanced due to its very low lysine level that the lysine it contains is not completely utilized. Maximum growth cannot be obtained by increasing the level of wheat gluten to provide 0.9 per cent lysine in the diet. However, if the lysine level is increased sufficiently to exceed the requirement slightly, either by increasing the level of protein (80 per cent wheat gluten) or by supplementing with lysine (to 1.2 per cent), a considerable excess of amino acids in the diet can be tolerated. In fact, wheat gluten, with a biological value of only 40, will support maximum growth if the level in the diet is increased sufficiently to provide about 1.2 per cent lysine.

From the data acquired in these studies, the authors have drawn an analogy between the rise in requirement for the most limiting amino acid as the dietary protein level is increased and the results of amino acid

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imbalance. Other investigators have suggested that the increased requirement for the limiting amino acid reflects: (1) increased anabolism (J. B. Allison, *Physiol. Rev.* **35**, 664 (1955)); (2) an alteration in the proportions of protein, fat and moisture in the tissues (H. H. Mitchell, *Ind. Eng. Chem. Anal. Ed.* **16**, 696 (1944)); and (3) an increased rate of amino acid catabolism (W. D. Salmon, *Arch. Biochem. Biophys.* **51**, 30 (1954)).

Thus the data of Munaver and Harper suggest that imbalance played a significant role in their experiments. However, the extreme complexity of the problem and the necessity of viewing each experiment within its own context indicate the need for further work before firm generalizations can be made. Therefore the caution of the authors in pointing out the analogy is entirely justified at this time.

### FURTHER STUDIES OF CHOLESTEROL, FATS AND FATTY ACIDS

*Varying levels of saturated and unsaturated fats, protein and cholesterol influenced the chemical composition of chicken aortas. Surprisingly, unsaturated fats as well as wheat germ increased serum cholesterol deposition in rabbits.*

So much is published respecting cholesterol and its biochemical interrelationships that it is difficult to know what facts are significant. Moreover, there is disagreement over the relative importance of diet, heredity and stressful environment in coronary vascular disease and atherosclerosis. The following studies, however, provide valuable information.

Since the quantity of cholesterol in the aorta has been used as a rough index of atherosclerosis, H. Fisher *et al.* (*J. Nutrition* **69**, 163 (1959)) studied the effects of different fats and of varying quantities of dietary protein, fat and cholesterol upon the chemical composition of the aortas of chickens.

In the first part of the experiment, day-old male chicks were given a normal ration for one week and then assigned a particular diet for ten weeks. The basal ration contained soybean meal (50 per cent protein), dextrose, vitamins and minerals, while two levels of protein (8 and 20 per cent), of corn oil (2 and 12 per cent) and of cholesterol (0.3 and 2.0 per cent) provided the experimental diet. After ten weeks on their respective diets, the chicks were sacrificed and blood was collected for determination of cholesterol and the aorta removed, cleaned and examined for degree of athero-

sclerosis. Aortic tissue was analyzed for cholesterol, polyunsaturated fatty acids, iodine number and hydroxyproline.

It was found that the lower intake of protein resulted in greater degrees of cholesterolemia irrespective of fat or cholesterol intake. Thus 0.3 per cent cholesterol and a low-protein diet resulted in greater hypercholesterolemia than 2.0 per cent cholesterol and 20 per cent protein in the diet, although the apparent degree of atherosclerosis of the aorta was greater in birds fed larger amounts of protein. The low-protein diet also resulted in aortic fats with a lower iodine number. It seems significant that the quantity of aortic cholesterol correlated negatively with the iodine number of aortic fat.

Visible atherosclerosis was more marked in the abdominal aorta than elsewhere. Analyses of these aortic tissues revealed that a high-protein diet increased the ratio of hydroxyproline to cholesterol and hence the degree of fibrosis.

In the second portion of the experiment, eight- and 20-month-old chickens were fed diets of varying degrees of saturation for one year, after which time the birds were sacrificed and their aortas examined. The chickens fed tallow in their diet grew to be heavier than those fed no fat or fed linseed

oil. Also, plasma and aortic cholesterol levels became higher in the older hens and they had a higher atherogenic score. Hydroxyproline content of the abdominal aorta increased with age and again the lesions were more severe in the abdominal aorta than in the aortic portions.

These data indicate that the abdominal aorta of the chicken is more susceptible to atherosclerosis and contains fats which are largely saturated. With age and with more abundant dietary protein the amount of fibrous material increases. Thus the cholesterol content of the plasma is not the only factor leading to atherosclerotic lesions since, in these studies, the aortas contained more hydroxyproline as they became more involved.

These investigators have related dietary fats and proteins to the development of atherosclerosis in the chicken. Large amounts of protein increase the degree of fibrosis and large amounts of fats lead to more cholesterol in aortic tissues, while dietary cholesterol seems to augment the ease with which lesions can be induced.

In an effort to compare the degree of atherosclerosis induced by hydrogenation of fats and that accounted for by degermination of cereals, E. Van Handel and D. Zilversmit (*J. Nutrition* **69**, 202 (1959)) conducted a study in rabbits. Three-month-old animals were fed diets containing approximately 300 calories per day. The basal ration was a commercial feed to which was added cottonseed oil, or hydrogenated cottonseed oil (respective iodine numbers 112 and 64). Other diets contained generous quantities of wheat germ or sucrose. These

four groups of animals were given 300 mg. of dietary cholesterol and a control group was given sucrose without cholesterol.

All five groups gained weight at approximately the same rate. Plasma concentrations of cholesterol increased over a period of five months in the animals fed either type of fat and in those fed wheat germ. However, the increase in cholesterol was modest in those fed sucrose with cholesterol, while those fed sucrose alone did not increase their cholesterolemia. Examination of their aortas revealed that atherosclerosis was minimal in the group fed sucrose, moderate in those fed sucrose and cholesterol as well as in those fed either of the two fats, and highest in those fed wheat germ. Hepatic cholesterol was high in both groups fed additional fat, but the amount of cholesterol in the liver did not correlate with that in the aorta. It is interesting that one group of litter mates, represented in each of the five dietary groups, failed to develop any aortic lesions.

This study provides an example of the complex nature of metabolic factors which regulate cholesterol metabolism. According to current concepts, the more hydrogenated fats should have increased both the serum cholesterol and the extent of aortic lesions, yet in this study natural oils had an effect comparable to those which had been hydrogenated. One also might expect that wheat germ, which contains vegetable protein, unsaturated fats, several vitamins and trace minerals, would protect the animals against atherosclerosis, but the opposite occurred. It is obvious that there is much to learn about the interrelationships of fats, proteins and cholesterol.

### GALACTOSE INGESTION AND URINARY EXCRETION OF CALCIUM AND MAGNESIUM

*Dietary galactose produces excessive urinary losses of calcium and magnesium. This effect does not depend upon rate of intestinal absorption.*

The lessons taught by study of rare diseases sometimes prove to be of great value in unexpected ways. At one time,

little concern was given to galactosemia, an inborn error of metabolism. Now, however, there is good reason to study this con-

dition in order to determine what effect the metabolism of galactose may have upon the normal individual.

Galactosemia may be classified into three groups. In its severe form an infant may begin to vomit, lose weight and become jaundiced. Soon the liver enlarges and ascites develops. As time progresses, the victim becomes lethargic, has diarrhea and may become blind due to cataracts. A less severe form of the disease may be recognized when the child is several months old. Here again symptoms and signs are similar; poor appetite, faulty rate of growth and hepatomegaly. Cataracts may develop as well. In the mild form of the disorder, however, the only clues may be an apparent distaste for milk and better rate of growth when the child begins to take solid foods.

In each of these three instances, the diagnosis depends upon the demonstration of an abnormal galactose tolerance test. Other abnormalities may include low levels of blood glucose and renal defects (galactosuria, proteinuria and aminoaciduria). Detailed studies were presented by A. Holzel, G. M. Komrower and V. Schwarz (*Am. J. Med.* **22**, 703 (1957)).

Little attention was paid to lactose or galactose until reports began to appear indicating that feeding these sugars to animals produced hypercalciuria (P. Handler, *J. Nutrition* **33**, 221 (1947); C. M. McCay *et al.*, *J. Gerontol.* **7**, 161 (1952)). A similar effect of dietary lactose on urinary excretion of magnesium was noted by J. Outhouse, J. Smith and I. Twomey (*J. Nutrition* **15**, 257 (1938)).

In an extension of this work, F. W. Heggeness (*J. Nutrition* **69**, 142 (1959)) studied the effects of feeding galactose to rats upon their ability to absorb and excrete magnesium and calcium. He fed a diet which contained 21 per cent casein, 14 per cent fat and 60 per cent of carbohydrate (either galactose or glucose) to white rats.

Their salt ration contained adequate calcium except in the special instances in which low calcium intake was desired. All animals were pair fed over seven day periods and balance studies were calculated for 24-hour periods.

The animals fed galactose gained weight slowly in comparison with those fed glucose, and they developed polyuria (with galactosuria) from the start. After about three weeks, cataracts began to develop. They also consumed much more water than did the control animals (60 ml. compared with 15 ml. per day).

Studies of intake and excretion of calcium revealed that rats fed galactose absorbed slightly more calcium than the rats fed glucose, but they excreted significantly more of this ion in their urine. Other rats fed a diet deficient in calcium but containing galactose also excreted more calcium than did those fed glucose. Thus, the hypercalciuria did not depend upon intestinal absorption.

Because antibiotics have been found to enhance intestinal absorption of calcium, Heggeness repeated the above studies with chloramphenicol added to the diets. This did result in significantly greater absorption of calcium by both groups of rats, but had no effect on the rates of urinary excretion of calcium.

Urinary losses of other cations were also studied. It was found that both sodium and potassium excretion were increased by galactose feeding, but did not parallel the temporal course of calciuria. Whereas increased calcium excretion occurred at once and continued unabated, sodium and potassium excretion increased several days later.

Magnesium excretion in the urine was almost twice as great in animals fed galactose as in those fed glucose. Dietary intake of this ion was approximately 0.6 mEq per 10 g. of food.

Despite the rapid rate of excretion, the

serum of rats fed galactose contained significantly more calcium than did that of the control animals. However, serum concentrations of magnesium did not differ between the two groups.

These results indicate that feeding galactose results in a negative calcium balance and presumably would result in demineralization of the skeleton. Depletion of bone ash was reported by A. B. Bonnamour and Escallon (*Compt. Rend. Soc. de Biol.* **74**, 1106 (1913)) who gave daily injections of lactose to rabbits for three months. Although bone ash was measured by Heggeness, his data could not be compared since the control

animals grew more rapidly than those fed galactose.

The mechanisms by which galactose increases intestinal absorption, raises serum concentration, and causes greater urinary excretion of calcium is obscure. However, it seems logical to examine further the possibility that renal tubular mechanisms may play a role.

This report must not be interpreted too literally. Nevertheless, it gives the incentive to study more closely the interrelationships which might be found between milk in the diet of adults and the occurrence of hypercalciuria, nephrolithiasis, and cataracts.

### AMINO ACID REQUIREMENTS FOR PROTEIN-DEPLETED CHICKS

*Chicks depleted of protein require more methionine and lysine than normal, but this is only a reflection of their greater need for protein.*

The concept that there is a fixed quantitative requirement of the dietary essentials has been challenged. In growing infants and children, N. S. Scrimshaw, R. Bressani, M. Béhar, and F. Viteri (*J. Nutrition* **66**, 485 (1958)) found evidence suggesting that the optimal quantities of amino acids may vary with the physiologic state of the subject. Moreover, Bressani, Béhar, Scrimshaw and Viteri (*Fed. Proc.* **17**, 471 (1958)) found that the addition of either tryptophan or lysine to the corn-masa diet of chickens with kwashiorkor would induce a positive nitrogen balance. This is in contrast to results with normal animals when the addition of either of two limiting amino acids will not only fail to improve nitrogen balance but will worsen it.

H. Fisher, P. Griminger and G. A. Leveille studied this problem in chickens under conditions of protein depletion (*J. Nutrition* **69**, 117 (1959)). They fed a diet containing 22 per cent of protein to day-old male chicks for two weeks, after which time half of the chicks were given a diet free of nitrogen for an additional week, while the other half were fed the stock diet. Next, the

chicks were grouped according to weight and fed graded levels of either of the two critical amino acids, methionine or lysine. Thus ten lots of ten chicks each received five different amounts of each amino acid in basal diets containing 20 per cent protein.

It was found that the chicks previously depleted of protein, derived added benefits from each increment of either amino acid. Meanwhile, the normal chicks had a plateau in rate of growth at the second level of amino acid supplement.

In an attempt to interpret these findings, the authors designed a further study in which normal chicks were fed diets containing amounts of protein successively smaller than the 20 per cent given in the original studies. This was to test the chicks' ability to utilize methionine or lysine only to the extent of their optional protein needs. By contrast, the depleted birds, requiring all of the protein supplied in the 20 per cent diet, might utilize these limiting amino acids to full advantage.

Accordingly, groups of protein-depleted and normal chicks were given diets containing varying amounts of protein (14 to 22

per cent for the controls and 16 to 24 per cent for the protein-depleted chicks). In half of each group, the optimal quantity of one limiting amino acid was added (for the controls, 1 per cent lysine or 5 per cent of the protein level, and for the protein-depleted chicks, 1.2 per cent lysine or 6 per cent of the protein level).

According to their hypothesis, normal chicks fed a diet containing somewhat less than 20 per cent protein, but including 1 per cent lysine, should gain weight as well as other normal chicks fed the higher level of protein. By contrast, the depleted birds should do less well if fed less than 20 per cent of protein even though lysine constitutes 1.2 per cent of their diet.

The results confirmed the validity of this hypothesis in both groups of birds. The normal birds required no more than 16 per cent protein when given 1 per cent lysine (6.2 per cent of the protein). Meanwhile, the depleted birds needed 20 per cent protein and 1.2 per cent lysine (6 per cent of the protein). Furthermore, their growth

could be accelerated by increments of protein, provided the lysine to protein ratio remained constant at 6 per cent.

These results indicate that chicks depleted of protein require larger quantities of methionine and of lysine than normal chicks, but this need is merely a reflection of their greater requirement for protein. In each instance, the amino acid requirement could be shown to represent a fixed per cent of the total protein requirement.

In clinical practice, instances of severe protein depletion are common, for example in patients with kwashiorkor, chronic nephritis and hepatic cirrhosis. It is possible that some of the problems encountered in correcting these protein deficiencies involve the basic principles set forth above. During the stage of protein repletion, the requirement of these patients for essential amino acids may be higher than anticipated, since their overall protein deficit may be huge. If the required ratio of amino acids to protein proves to be constant in humans, as it is in chicks, this information may be of significant value in clinical practice.

### COLD AS A LIPOTROPIC AGENT

*Rats maintained at 1°C show decreased liver triglyceride accumulation on a high-fat diet but increased liver cholesterol esters when fed a high-cholesterol diet.*

Studies of the various nutritional requirements of different climates are of practical importance since many of the areas of most desirable climate are becoming overpopulated. For example, does the apparent preference for high-fat diets of those subjected to very low temperatures have a physiological basis? There is considerable evidence to suggest that it does.

In rats maintained at 1°C, efficiency of protein utilization (on a low-protein diet) has been found to be at a maximum with 40 per cent dietary fat (*Nutrition Reviews* 16, 188 (1958)). Moreover, even on this high-fat diet, fatty livers did not develop at the low

temperature. That cold acts as a typical lipotropic agent in both prevention and cure of fatty livers has been demonstrated by C. R. Treadwell, D. F. Flick and G. V. Vahouny (*Proc. Soc. Exp. Biol. Med.* 97, 434 (1958)).

In a continuation of this study, Vahouny, Flick, H. M. Gregorian and Treadwell (*J. Nutrition* 68, 495 (1959)) have carried out experiments designed to extend the knowledge gained with triglyceride fatty livers to cholesterol fatty livers. The diet used to promote a deposit of cholesterol esters in the livers of young male rats consisted of 20 per cent casein, 20 per cent

fat (18 per cent lard plus 2 per cent cod liver oil), 25 per cent each of starch and sucrose, 5 per cent salt mixture, 1 per cent sodium taurocholate and adequate amounts of micronutrients with the exception of inositol and choline, which were omitted. The experimental diet contained, in addition, 2 per cent cholesterol.

In the first experiment (preventive), groups of rats were maintained for 21 days on each diet at 1°C and 25°C. The rats kept at 1°C had 25 to 40 per cent greater caloric intake but gained only half as much weight. Moreover, protein efficiencies were greater at the higher temperature. Survival at the low temperature was 100 per cent on the control diet and 87 per cent on the cholesterol-containing diet.

At the end of the 21-day period, all the rats which had been kept at 1°C and a third of those at 25°C were used for chemical studies while half the remainder were changed to the lower temperature for a curative study. The change in temperature resulted, as before, in increased food intake and greatly decreased growth rate and protein efficiency. After a further 21-day period, all rats were used for chemical studies.

As had been noted previously, the liver-fat content of rats fed the control diet at 1°C was significantly lower than in those fed the same diet at 25°C, thus confirming the lipotropic effect of cold for triglyceride fatty livers. However, the liver lipid of animals on the cholesterol-containing diet was much higher than that of controls and those of the low-temperature animals were, if anything, higher than those of the 25°C animals.

Analysis of the liver lipids furnished an explanation for these observations. In the preventive experiment, the effect of cold was to greatly decrease the per cent of triglyceride of rats on the control diet (from about 56 to 18 per cent) and increase phospholipid (from 31 to 67 per cent), thus

confirming a more efficient fat catabolism at lower temperatures. This same effect was noted for rats on the cholesterol-containing diet, but in this case cholesterol esters had risen from about 10 per cent to 55 per cent at 25°C and to about 65 per cent at 1°C. It was thus evident that cold did not aid in the catabolism or removal of cholesterol esters.

In the curative experiment, the same effect was noted. Liver triglyceride was lowered and phospholipid was raised by cold, whereas when the diet contained cholesterol the per cent of cholesterol esters was even higher in the 1°C animals than in those of the higher temperature group. Further insight into the effect of temperature on cholesterol was obtained by examination of the blood lipids during both experiments. Although blood cholesterol was greatly elevated in all animals on the cholesterol-containing diet, it was significantly greater at the lower temperature.

These studies confirm previous observations that cold acts as a true lipotropic agent, preventing as well as curing fatty livers induced by a high-fat diet in the absence of adequate dietary lipotropic agents. They further show that cold has neither a preventive nor curative effect on the accumulation of liver cholesterol esters induced by a high-fat high-cholesterol diet. The antilipotropic effect of cold on the "cholesterol" fatty liver was independent of its effect on the "triglyceride" fatty liver and was not readily explainable by known mechanisms. Since no accumulation of liver cholesterol was noted on the low-cholesterol diet, it is possible that the effect of cold was to increase absorption of dietary cholesterol or to decrease its inhibiting effect on liver cholesterol synthesis.

In any event, these findings, if applicable to man, should have a considerable influence on formulation of diets for those who must adapt, even for relatively short periods, to a subnormal temperature



## ALCOHOL AND EXPERIMENTAL ATHEROSCLEROSIS

*Alcohol was fed to cockerels on atherogenic diets in order to determine the effect on plasma lipids and on aortic and coronary atherosclerosis.*

At one time there was a widespread belief that arteriosclerosis was the direct result of excessive alcohol consumption, while at present there is a prevailing view that alcohol consumption may prevent or even reverse the arteriosclerotic process. Neither of these positions is well supported by existing evidence. Some have suggested that alcoholic liver disease may lead to a defect in estrogen catabolism and that the hyperestrogenism may have a beneficial effect on arteriosclerosis in some patients.

Autopsy data derived from alcoholic subjects appears to be controversial. S. L. Wilens (*J. Am. Med. Assn.* **135**, 1136 (1947)) in a comparison of chronic alcoholics with moderate drinkers and abstainers could find no differences in the degree of atherosclerosis. H. A. Edmondson, E. M. Hall and R. O. Myers (*Alcoholism*, G. N. Thompson, Editor. Charles C. Thomas (1956)) believed that development of coronary atherosclerosis in their subjects was inhibited by alcohol. However, their study suffered from lack of adequate control material.

On the other hand, N. Kimura (*cited by A. Keys, Minnesota Med.* **38**, 28 (1955)) found that in his subjects atherosclerosis was more advanced in men using alcohol and tobacco compared with men using neither. Victims of myocardial infarction, when compared with a healthy control group, consumed less alcohol but more concentrated alcoholic beverages according to a study by M. M. Gertler *et al.* (*J. Am. Med. Assn.* **146**, 1291 (1951)). Moreover, D. M. Spain and V. A. Bradess (*Arch. Int. Med.* **100**, 228 (1957)) found that 7 per cent of men dying of coronary occlusion had a history of chronic alcoholism. Thus these authors did not support the concept that alcohol protected against atherosclerosis.

In view of the difficulty in evaluating the effect of alcohol ingestion on atherosclerosis in man, E. Nikkila and O. Ollila (*Circulation Res.* **7**, 588 (1959)) have undertaken controlled studies with experimental animals. Leghorn cockerels seven weeks of age were divided at random into six groups of 35 animals per group and fed three different diets with and without alcohol. Commercial chicken feed only was given to the first two groups. Cholesterol (1.5 per cent) was added to this diet for the next two groups, and for the last two groups 1.5 per cent cholesterol was added to a low-protein diet. For those groups receiving alcohol, a 7 per cent solution was substituted for drinking water and the intake adjusted so that blood levels averaged about 50 mg. per cent. The intake of paired diet groups was identical.

During the 18 weeks of the experiment 37 animals died and were excluded. Venous blood samples drawn after fasting were analyzed before the start of the experiment and at the end of five, 11 and 18 weeks. At the end of the period, the birds were sacrificed and heart and aorta were evaluated grossly and microscopically. The most difficult problem was the grading of the atherosclerotic lesions. They employed, in modification, a method proposed by E. J. Orme (*Acta Physiol. Scandinav.* **41**, suppl. 142 (1957)) who, criticizing the non-linearity of some older techniques, had proposed a "descriptive semi-quantitative" classification. Aorta and iliac arteries were evaluated, and lesions in the thoracic aorta and brachiocephalic arteries were graded by such descriptions as "intact," "slight yellowness," and "marked yellowness."

Chemical analyses included total plasma cholesterol and alcohol concentration. After



electrophoretic separation of alpha- and beta-lipoprotein into ten random samples, the amount of cholesterol was determined in each fraction.

Total cholesterol averaged between 106 and 110 mg. per cent before the experiment started. During the experiment, the cholesterol values gradually rose to 126 mg. per cent in the group fed the unaltered diet and water while in the group receiving this diet plus alcohol they rose to 158 mg. per cent. The cholesterol-fed groups showed a marked initial rise in cholesterol values during the first five weeks, followed by a fall by the eleventh week and another rise toward the end of the experiment. The cholesterol-fed birds that drank only water had plasma cholesterols averaging 312 mg. per cent, while the birds receiving alcohol averaged 376 mg. per cent. Animals fed the cholesterol-supplemented, low-protein diet and water had a cholesterol level of 309 mg. per cent at 18 weeks, whereas the alcohol-drinking birds averaged 605 mg. per cent.

Plasma phospholipid levels were significantly higher in the alcohol-drinking birds fed the unaltered or low-protein diet.

Analysis of lipoproteins revealed that in all groups the alpha-lipoprotein cholesterol was essentially unaffected and that the increase in cholesterol was solely in the low-density lipoprotein class.

Comparison of the severity of atherosclerosis in thoracic aorta and coronary arteries in the various groups showed that alcohol ingestion did not influence either the incidence or severity of atherosclerosis. The control group showed less atherosclerosis than either cholesterol-fed group, but it made no difference whether a group was fed water or alcohol.

If the grading scale is considered to be linear, the plot of the grade of atheromatosis against the plasma cholesterol in milligrams per cent, plotted on a logarithmic scale, gives regression lines which in turn show that the difference between the slopes

is not significant and that alcohol does not inhibit deposition of lipid.

All animals of the first and second groups showed normal liver histological structure. On the other hand, the low-protein birds had vacuolated liver cells and diffuse cell boundaries, and the ethanol-fed group did not differ from the group fed water.

Although the high plasma cholesterol was correlated with atherosclerosis in the cholesterol-fed animals, the accumulation of lipids in the aorta did not depend on this variable alone. In fact, the animals in this study developed similar arterial lesions with widely different plasma cholesterol levels. Likewise, the alloxan-diabetic rabbits of G. L. Duff and G. C. McMillan (*J. Exp. Med.* **89**, 611 (1949)) and the estrogen-treated chickens of J. Stamler *et al.* (*Circulation* **6**, 460 (1952)) also showed atheromatous responses not foreshadowed by the serum cholesterol level.

In the present study, while chronic alcohol ingestion appeared to promote hypercholesteremia, it may also have protected the arteries against the damage usually expected from extreme cholesterol elevation. The serum cholesterol differences between groups remained statistically insignificant since the plasma cholesterol levels varied greatly within a single severity class of atherosclerosis.

Further study is needed to determine the mechanism by which alcohol increases the levels of serum cholesterol and phospholipid. The experiments of M. D. Siperstein and V. M. Fagan (*Science* **126**, 1012 (1957)) have demonstrated the regulation of cholesterol and fatty acid synthesis by pyridine nucleotide coenzymes. Since the metabolism of alcohol is dependent on DPN, this may suggest a possible metabolic relationship between cholesterol synthesis and alcohol.

Further work will also be necessary to determine whether the method used in grading lesions is really sensitive enough to be used in a study of this type.

## SITE OF ACTION OF LACTOSE ENHANCEMENT OF CALCIUM UTILIZATION

*Lactose acts by enhancing calcium absorption from the intestine. Neither lactose nor a lactose metabolite act directly on the bone cell.*

The mechanism for improved calcium absorption occasioned by the inclusion of lactose in the diet remains unknown. However, a series of experiments in rats carried out by F. W. Lengemann (*J. Nutrition* **69**, 23 (1959)) have led him to conclude that lactose acts in the digestive tract and not at some site within the body, as previously suggested by P. Fournier (*Compt. Rend. Acad. Sci.* **239**, 718 (1954); **240**, 1364 (1955)) and by Fournier *et al.* (*J. Physiol. (Paris)* **47**, 351 (1955)).

Lengemann, R. H. Wasserman and C. L. Comar (*J. Nutrition* **68**, 443 (1959)); *Nutrition Reviews* **18**, 115 (1960)) have reviewed previous work on this topic and studied the enhancement by lactose of  $\text{Ca}^{45}$  and  $\text{Sr}^{85}$  absorption in the rat.

In the present studies, Yale-Wistar male and female albino rats raised and maintained on a dog chow were fasted for 24 hours and administered test solutions while the animals were under light ether anesthesia. After an additional 24-hour fast, the rats were sacrificed, their femurs removed and the radioisotope content of the femurs determined (Wasserman, Comar and M. M. Nold, *J. Nutrition* **59**, 371 (1956)). The results were expressed as the percentage of administered dose contained in the two femurs.

In the first experiment, rats were given orally 0.13 millimole of radiocalcium and orally or intraperitoneally 1 millimole of lactose. Another group received radiocalcium intra-abdominally and were compared with another group receiving both lactose and radiocalcium by the same route. The data acquired demonstrated that lactose placed in the intestinal tract had a remarkable ability to stimulate calcium absorption but none when administered intra-abdominally. The femur uptake by

animals given radiocalcium intraperitoneally was not increased significantly by lactose.

In the second experiment, rats were given daily intraperitoneal injections of saline, glucose or lactose for two weeks before receiving the test oral dose of radiocalcium after a 24-hour fast. The data showed that long-term predosing with lactose had no effect on the uptake of radiocalcium from the intestinal tract by the femur. When rats were given radiocalcium intraperitoneally 24 hours prior to the start of a two-week period of daily intraperitoneal saline, glucose or lactose injections, no statistically significant differences between groups existed respecting removal of radiocalcium from the femurs. This implies that lactose within the body has no effect on the removal of calcium from the skeleton.

To eliminate the possibility that a metabolic product of lactose, possibly produced in the lumen of the walls of the intestine, could affect bone cell metabolism, a third experiment was performed in which 0.065 millimole of calcium labeled with  $\text{Sr}^{85}$  (Lengemann, Wasserman and Comar, *J. Nutrition* **68**, 443 (1959)) was placed in a ligated segment of the ileum of the rat and 0.25 millimole of lactose placed in an adjacent portion. Control animals received  $\text{Sr}^{85}$ -labeled calcium alone or a mixture of lactose and  $\text{Sr}^{85}$ -labeled calcium. The data on uptake of  $\text{Sr}^{85}$  by the femur showed that lactose was effective only when it and calcium were present in the same segment, implying that no metabolite was involved.

Since the conditioning of a bone cell by a theoretical metabolite might require time, a fourth experiment was performed in which rats were fed for two weeks a stock ration to which either 10 per cent (by weight) of glucose or lactose had been added. The animals were fasted 24 hours and given oral

doses of radiocalcium to which either glucose or lactose had been added. Prolonged lactose feeding had no effect on calcium uptake by the femur when glucose was included in the test solution, thus supporting the evidence from the previous experiment that no metabolite of lactose was affecting bone cell metabolism. Interestingly, the response to lactose by rats preferred glucose equalled that of rats preferred lactose, implying that the development of an intestinal flora capable of attacking lactose was not essential for the enhancement of calcium absorption. This also indicated that the enhancing effect could be sustained over at least a two-week period.

A final group of experiments indicated that the enhancing effect of lactose was not limited to calcium but was general for the members of group IIA ( $Mg^{28}$ ,  $Sr^{85}$ ,  $Ba^{133}$  and  $Ra^{226}$ ) of the periodic table. Lysine acted in a fashion similar to lactose (Wasser-

man, Comar and Nold, *J. Nutrition* **59**, 371 (1956)) except that it did not enhance the absorption of magnesium, suggesting that the modes of action of lactose and lysine may differ.

The author has done an effective job in demonstrating that lactose per se in the tissues of the rat had no effect on absorption of calcium from the intestine or the distribution of absorbed calcium and that for two weeks lactose was unable to prime any possible mechanism which would enhance calcium utilization. Thus his data suggest that lactose acts primarily in the digestive tract. As in the case of many other nutrients, the importance of investigating further the role of the small bowel in absorption and, in some instances, metabolism is emphasized. Although the difficulties of studying the small bowel *in vitro* and particularly *in vivo* are great, work such as this suggests that the rewards of surmounting these difficulties would be great.

## METABOLISM OF SOME IODINE-CONTAINING COMPOUNDS IN CATTLE

*The biological half-life of thyroid hormones, thyroxine and triiodothyronine (about 2.5 days) results in the build-up of a body pool. Dietary factors influence the level of plasma protein-bound iodine.*

The association of thyroxine secretion with milk production, egg production and growth rate has led to a continuing interest in the pattern of thyroid hormone utilization by animals. Factors influencing the activity of the thyroid have been studied by B. N. Premachandra, G. W. Pipes and C. W. Turner (*J. Dairy Sci.* **41**, 1609 (1958)), who observed that during the summer the secretion rate of the thyroid is about one-third that which prevails during the winter. These same investigators have recently pointed out that, since thyroxine and triiodothyronine are metabolized in the somatic cells of the body, the biological half-life and turnover rates are of special importance (*Ibid* **42**, 1606 (1959)).

It has been shown that triiodothyronine,

which is formed by the removal of one iodine molecule from thyroxine, may be as active or more active than thyroxine (J. Gross and R. Pitt-Rivers, *Biochem. J.* **53**, 652 (1953)). The further deiodination of triiodothyronine will result in inactive compounds, but the iodine which is released may be picked up by the thyroid or it may be secreted in the urine. Thyroxine itself may be excreted in the bile and reabsorbed from the intestinal tract. With these alternatives, the biological half-life of L-thyroxine and L-triiodothyronine is of great physiological importance because it establishes the level of biological activity of these compounds in the animal body.

Presumably, if the rate of disappearance of thyroxine is extremely rapid, then the rate of

thyroid secretion would represent the effective blood thyroxine level. In this case, the picture would be similar to that with some of the steroid hormones (W. H. Pearlman, *Ciba Foundation Colloquia on Endocrinology* 11, 233 (1957)). On the other hand, elimination of only part of the thyroxine secreted each day would permit an accumulation of thyroxine and triiodothyronine in the blood and tissues, and the biologically effective pool in the body would be greater than the daily thyroxine secretion rate.

By use of  $I^{131}$ -labeled thyroxine and triiodothyronine, Pipes, Premachandra and Turner (*loc. cit.*) studied the biological half-life of these compounds in mature Guernsey and Jersey cows. To prevent recirculation of metabolized iodine from the thyroxine and triiodothyronine, thiouracil was administered daily to serve as a thyroid block. They administered between 200 and 400 microcuries of labeled compounds and assumed that the disappearance of radioactivity from the blood represented the amount of thyroxine  $I^{131}$  which had been excreted as such, or deiodinated, and the  $I^{131}$  excreted.

From these data the rate of metabolism of thyroxine was computed and the biological half-life estimated by extrapolating to zero time from the observed values. It was apparent that, in contrast to the effect of temperature on thyroid secretion, different temperatures did not significantly influence the biological half-life or the turnover rate of thyroxine. Thus similar biological half-life values of 2.54, 2.31, 2.65 and 2.41 days were obtained in spite of average daily temperatures of 47°, 53°, 71° and 73°, respectively. The corresponding turnover rates were 27.6, 30.1, 26.7 and 29.2 per cent.

There appears to be a significant difference in the biological half-life and turnover rate of triiodothyronine and thyroxine. In March of 1958 when the study was being carried out on thyroxine, a study was also carried out on triiodothyronine  $I^{131}$  which demonstrated a biological half-life of 1.99 days and a

turnover rate of 35.3 per cent for this compound.

It may be concluded that, as a result of the relatively slow metabolism and secretion of thyroxine, there is a build-up of thyroxine in the blood and total body space to a level significantly higher than the daily thyroxine secretion rate. When there is constant daily thyroxine secretion, varying according to temperature, the body thyroxine pool changes correspondingly. With increased thyroxine secretion, the body pool increases slowly, whereas when thyroxine secretion decreases, the pool rapidly declines.

These observations are of particular importance in instances where thyroprotein is fed to animals to increase the effective level of thyroid activity. The authors present an example in which animals fed 15 g. of thyroprotein per day required 22 days to reach apparent equilibrium when they secreted 5 mg. of thyroxine per day per 1000 pounds of body weight with an effective body thyroxine pool of 38.6 mg. On the other hand, complete withdrawal of thyroprotein returned the body pool to its previous level of 12.7 mg. in 3.9 days.

This observation undoubtedly accounts for the precipitous drop in milk yield which occurs when thyroprotein is suddenly withdrawn from the diet. The time lag in resumption of thyroxine secretion is probably directly responsible for the drop in mammary gland activity.

R. O. Asplund, G. A. McLaren, H. O. Henderson and I. D. Porterfield (*J. Dairy Sci.* 42, 1718 (1959)) observed plasma protein-bound iodine (PBI) levels in cattle which were considerably above those generally regarded as normal. These high values were all the more significant since they did not result from a high-iodine intake nor from any known substances possessing thyroid activity. In this respect, their study differed from earlier reports of J. F. Long *et al.* (*J. Dairy Sci.* 35, 503 (1952)) and Long, L. O. Gilmore and J. W. Hibbs (*Ibid.* 39, 1323 (1956)), who observed elevated PBI values when either iodinated casein or

potassium iodide was fed to cows. A similar effect was observed by J. P. Mixner and H. D. Lennon, Jr., (*Ibid.* 41, 840 (1958)) when thyroid hormones were used in the treatment of young bulls.

In the study by Asplund and co-workers, the abnormally high PBI values always occurred during late winter and spring of each year. The values varied much more in calves than in cows, while the extremely high values (45  $\mu$ g. per cent) were found only in the calves. The periods of elevation were shown to have a regular pattern; in both 1957 and 1958 the values reached a peak in March and then declined.

These investigators separated the plasma PBI into the active (*T*) and inactive (*D*) fractions and found no evidence of a patho-

logical thyroid state, although the thyroactive fraction was much higher than normal. Interestingly enough, while the high plasma PBI values (20 to 25  $\mu$ g. per cent) corresponded very closely to the period when Ladino clover and grass silage were fed, these workers suggest that the silage was probably only one factor contributing to the elevated PBI values.

Both of the above studies indicate the importance of environmental factors and of dietary constituents in the function of thyroid and of thyroid hormones. The close relationship between thyroxine secretion and milk secretion emphasizes the need for critical evaluations of the mechanisms governing thyroxine and triiodothyronine function.

## BODY COMPOSITION AND ENERGY METABOLISM

*Variations in body fat occur in rats of uniform weight when weight is increased under uniform conditions. During rapid growth, composition of weight gained can be calculated from respiratory data.*

An increasing amount of work is being directed toward the determination of gross body composition. This is true both for animals and man (*Nutrition Reviews* 14, 45, 98, 267, 298 (1956); 16, 14, 199 (1958)).

Although some of the previous techniques could be used in evaluating the composition of body weight changes (A. Keys *et al.*, *The Biology of Human Starvation*, vol. 1, p. 84. Univ. Minn. Press (1950)), there have been practically no studies in which these changes in body weight have been validated by actual analyses.

An interesting method for evaluating the changes in body composition has been presented by A. W. Pratt and F. K. Putney (*Am. J. Physiol.* 197, 660 (1959)) of the National Cancer Institute. They calculated the composition of intact rats from the data secured in an energy and nitrogen balance study carried out in an ingenious respirometer (*J. Nat. Cancer Inst.* 20, 161 (1958)). The body composition calculated by this

procedure at the end of the experiment was validated by carcass analyses.

As soon as their rats reached weights of 80 to 90 g. they were put on a purified diet of casein, corn starch, sucrose, hydrogenated vegetable oil and all the essential minerals and vitamins. Groups of rats were sacrificed when they attained weights of 150, 200, 250 and 300 g. respectively. The gastrointestinal contents were removed from the carcasses which were then ground and dehydrated to constant weight by lyophilization. The dry residue was ground and analyzed for nitrogen, lipid and caloric content (the latter by means of the bomb calorimeter).

The results indicated that within each weight group, the weights of the cleaned carcasses were fairly uniform. There was, however, considerable variation in the composition of the carcasses. The greatest variation occurred, as one would expect, in the fat content. For instance, in the rats weighing 200 g., the fat in the dried carcass

ranged from 30 to 43 per cent. This variation appeared in spite of the homogeneity of genetic background, the uniformity in diet and environmental conditions under which the animals were raised.

The fat-free carcasses showed little variation in composition except for water, which decreased from an average value of 78 per cent in the 150 g. rats to 74 per cent in the 300 g. animals. The nitrogen in the dried fat-free carcasses ranged from 134 to 140 mg. per g. with an average of 137. Similar variations in nitrogen content were seen in each weight group. When nitrogen values were translated into percentages of protein ( $N \times 6.25$ ) and corrected for the moisture, the fat-free tissue showed an increase in protein from 18.8 to 22.3 per cent as the animals went from a body weight of 150 to 300 g. There was a greater variation in ash content both within each weight group and for all animals. The ash content of the dried, defatted carcasses ranged from 135 to 160 mg. per g. with an average of 148.

The caloric content of the dried, fat-free tissue ranged from 4.5 to 4.7 Kcal per g. with an average of 4.65; that of the extracted fat ranged from 9.2 to 9.4 with an average of 9.28 Kcal. The extracted fat contained less than 1.5 mg. per g. of nitrogen.

The nitrogen content of the dried carcass was plotted against its caloric value. When this was done for all 16 animals, a surprisingly straight line resulted. Since it was felt that the values for the two extremes (extracted fat and fat-free tissue) could be determined with the greatest accuracy, they were used in plotting the relationship. This straight line relationship suggests that the dried carcass can be considered a mixture of fat and nonfat, thus permitting the addition of the values for per cent of fat along the axis for nitrogen content. The values for fat, of course, decrease as the nitrogen content increases.

Carcass analyses carried out on other

rats that had been raised on commercial stock or purified diets provided data which fit the above straight line relationship fairly well. Replicate analyses indicated that the error of measurement for the nitrogen determination was 1.25 mg. per g. while that for the bomb calorimeter was 0.05 Kcal per g. These errors are smaller than the observed deviations of individual animals from the straight line, suggesting that there are probably unexplained variations in body composition that account for the deviations.

To measure the caloric value of the tissue gained during growth, five male rats were kept for eight days in the respirometer. From the oxygen consumed by the rats and the water and nitrogen retained, the caloric value and the nitrogen content of the weight gained was calculated. The weight gains of these rats ranged from 36 to 64 g. for the eight days. When the values for the caloric and nitrogen contents of each gram of weight gained were compared with the above mentioned straight line, it was observed that the fat contents of the weight gains ranged from 15 to 41 per cent.

This procedure was validated primarily on circumstantial evidence. One such bit of evidence is the fact that the calculated water content of the weight gained was less than the lowest percentage of water found in the fat-free mass of the whole animal. This would occur necessarily, since the moisture content of the fat-free carcass decreases with increase in body weight.

The use of energy balance data as a means of computing the composition of the weight gained or lost by the animal during a period of observation in a respirometer is an interesting projection of a technique that is being used more frequently. With the increasing emphasis on energy metabolism, the paper by Pratt and Putney should serve as a stimulus for the elaboration and extension of the determinations that are secured in respiratory and balance studies.



## NOTES

### Gordon Research Conference

The 1960 Gordon Research Conference on Food and Nutrition will be held August 8 to 12 Colby Junior College, New London, New Hampshire. Topics scheduled for discussion include the following:

August 8—The use of a chelating agent in food processing; characterization of flavors by gas chromatography; radioactive tracers for screening new food additives for safety; the new regulatory control of food additives.

August 9—Correlation of biochemical measurements with other parameters of nutritional status; correlation of clinical observations with nutritional status; interpretive guides for nutritional appraisal; dietary survey techniques and interpretation.

August 10—Amino acid imbalance; amino acids in blood plasma as an indication of biological value of proteins; use of water-soluble, chemically defined diets in studies of nonessential amino acids and *in vivo* metabolic processes; food technology and the availability of dietary amino acids.

August 11—Evidence for the prevention of atherosclerosis in man by dietary means; lack of evidence for the prevention of atherosclerosis in man by dietary means; experimental production of severe protein malnutrition.

August 12—Effect of processing on the properties of dietary fats; nutritional and metabolic effects of radiation.

Requests for attendance at the conferences, or for additional information, should be addressed to W. George Parks, Director, Department of Chemistry, University of Rhode Island, Kingston, Rhode Island. After June 13 Dr. Parks' address will be Colby Junior College, New London, New Hampshire.

### Oxalate Formation in Hyperoxaluria

In the bodies of animals oxalate can be formed from glyoxylate, which is one of the compounds produced in the metabolic degradation of the amino acid, glycine. Patients who have a disease called primary hyperoxaluria normally excrete large amounts of oxalate in the urine (100 to 400 mg. per day), and the resulting precipitation of calcium oxalate crystals leads to kidney damage. Prognosis for such individuals is not good and they usually die in childhood or early adult life from either kidney failure or the effects of hypertension.

Recently, J. C. Crawhall, E. F. Scowen, and R. W. E. Watts (*Lancet* II, 806 (1959)) have studied the role of glycine as a precursor of urinary oxalate in both patients with hyperoxaluria and a normal individual (Crawhall, R. R. DeMobray, Scowen and Watts (*Ibid.* II, 810 (1959))).

The subjects were given isotopically labeled glycine with the tagged carbon in the carboxy position; the stable isotope  $C^{13}$  was used. These investigators studied the conversion of glycine in the first glycine metabolic pool (that pool into which a dose of glycine is immediately mixed after absorption and distribution and can be estimated by the amount of isotope in the uncombined urinary glycine).

They concluded that from 32 to 50 per cent of the urinary oxalate in patients with hyperoxaluria was derived from glycine of the first metabolic pool, while in the single normal individual, 40 per cent of the urinary oxalate was derived from the glycine pool. Thus these investigators believed that about one third to one half of the urinary oxalate was derived from glycine, and that this conversion probably occurred by way of glyoxylate. In patients with hyperoxaluria, the isotope dilution studies indicated that about

the same proportion of urinary oxalate is being derived from glycine as for the normal subject.

The authors believe that the fundamental defect in hyperoxaluria is probably a failure to degrade glyoxalate rather than an increased rate of production. If there were an increased rate of metabolism of glycine by way of glyoxalate, one would expect a high urinary oxalate excretion with a larger isotope incorporation in the patient with hyperoxaluria than in the normal subject. Thus the investigators draw the tentative conclusion that the primary error is a failure to degrade glyoxalate in the normal manner.

### Coronary Disease and Serum Lipids

Numerous reports have demonstrated that the average values for the serum concentration of cholesterol and several lipids are higher in groups of patients who have coronary disease than they are in normal groups of persons. In a recent study, W. R. Scarborough, E. W. Smith and B. M. Baker, Jr., (*Am. Heart J.* **59**, 19 (1960)) demonstrated that serum levels of cholesterol, alpha-2-globulin and beta-lipoprotein, and ballistocardiographic tracings correlated with age and coronary heart disease. They studied 165 normal subjects and 115 patients with coronary heart disease (212 men and 68 women), ranging from 20 to 30 years of age. Values of serum cholesterol, alpha-2-globulin and beta-lipoproteins were plotted in distribution curves according to the age and sex of the patients.

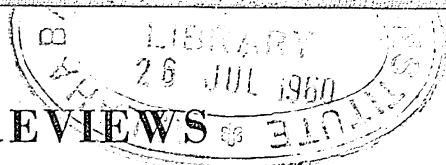
Ballistocardiograms were performed and were classified as normal, borderline or abnormal.

The authors found that, normally, the serum cholesterol rose progressively with age, reaching a maximum in the seventh decade and declining thereafter. A similar trend was found in patients with coronary disease, but the values were significantly higher and reached a maximum at an earlier age. Changes in the lipoproteins were found to vary widely, but abnormalities of beta-lipoprotein and of alpha-2-globulin were considerably more frequent in the presence of coronary heart disease. Moreover, all of these factors became abnormal more frequently with increased age.

This study demonstrated that the serum cholesterol and the alpha-2-globulin and beta-lipoproteins correlated significantly with increasing age and with coronary disease. Correlation between an abnormal ballistocardiographic tracing and these chemical determinations was doubtful.

### 1959 Build and Blood Pressure Study

Volume I of the 1959 Build and Blood Pressure Study, prepared by the Committee on Mortality under Ordinary Insurance and Annuities, was published last October. It contains a description of the findings of the study and summary tables. Volume II of this study is to appear shortly and will contain detailed tables. Order requests should be addressed to Society of Actuaries, 208 South La Salle Street, Chicago 4, Illinois.



## BIASED CRITICISM OF FLUORIDATION

Public health measures of any sort should be able to stand up to searching criticism; in fact it is a matter of great interest to the public that such criticism should exist and should be met. It is only reasonable to expect, however, that the criticism shall be fair-minded, shall present mistakes or inadequacies in their proper relationship to correct and adequate accomplishments, and shall not present conclusions to the public which are unwarranted by the evidence. The occasion for these remarks is the publication of an 83-page booklet, *Fluoridation, Errors and Omissions in Experimental Trials* by Philip R. N. Sutton (Melbourne University Press, Victoria, Australia, 1959, available through Cambridge University Press, New York).

Dr. Sutton, a Senior Research Fellow at the University of Melbourne Dental School and apparently without field experience in the epidemiological study of fluoridation, has devoted himself to a critical analysis of the printed reports of the long-term pilot studies of water fluoridation made in Grand Rapids, Michigan; Evanston, Illinois; Brantford, Ontario; and Newburgh, New York. The result is pure destructive criticism. A few errors and omissions are pointed out which might have been avoided. There are two or three valuable suggestions for future pilot studies of a similar nature. The bulk of Sutton's material, however, comprises criticism of a minor or irrelevant nature with no attempt to appraise the large positive accomplishments and extraordinarily comparable results of the four fluoridation projects. On frequent occasions he makes the mistake of trying to answer with a categorical "yes" or "no" questions which are far too complex for such black-and-white treatment. Such thinking also finds its way into his final conclusion that, "The sound basis on which the efficacy of the public

health measure must be assessed is not provided by these . . . crucial trials."

Sutton's criticisms deal chiefly with four of the more important aspects of project design: adequacy of controls, randomization of sampling procedure, adjustment of sample size to compensate for variability in caries attack rate, and variability or bias of examiner. Each of these deserves separate attention.

In discussing requirements for a control, Sutton adopts the position that the control city should be "comparable in all respects" to that where fluoride is being added. Thus he criticizes the selection of Kingston as a control for Newburgh because there are differences in calcium, magnesium and hardness of the water between the two cities. It does not seem to concern him that only with the third of these characteristics is there even a hint of evidence that it may be related to caries attack rate, nor that even if a relationship were known to exist in all three characteristics the matter would be irrelevant if the balance of other factors brought it to pass that initial caries rates were roughly similar in the two cities before fluoridation, which was indeed the case.

He expresses similar concern over the possible climatic and other differences between pilot and control cities, harking back to the caution quite correctly shown by H. T. Dean and other early students of the caries-fluorine hypothesis, when attempting to compare different endemic levels of dental caries in cities where fluoride level also differed. At this point, of course, it was very necessary to realize the possible existence of causative factors other than the fluoride in the water. Such caution is not necessary where the control city passes all reasonable tests for comparability including that of a similar initial caries rate, and the experiment involves a known environ-

mental change in the study city. Strict observance of Sutton's desires on selection of a control city would make epidemiological study virtually impossible.

Another criticism which deals with adequacy of control concerns the number of examinations made in each control city. Minor fluctuations in caries rate in the various control cities are made a matter of criticism even though these fluctuations do not contradict the general trend toward steady caries rates in the control cities and dropping caries rates in the pilot cities. Muskegon, Michigan, is criticized as a control city because fluoridation was instituted there three years before the end of the Grand Rapids study period. No consideration is given to the fact that within three years the demonstrable results of fluoridation are extremely small and are confined to certain very young age groups, nor to the fact that what influence may occur is in the direction of reducing differences in caries rate from a previously fluoridated city. Thus the differences which remain achieve added significance.

Sutton apparently has a fixation on the subject of random sampling, and is not familiar with the principle of stratified sampling designed to handle situations where randomization alone could produce biased results. Thus he criticizes the selection of representative schools in the large city of Grand Rapids and the examination of "almost all schools" in Muskegon, a smaller city. Carrying this thought further, he criticizes the Evanston study because white children in the control area (Oak Park) were compared with white children in Evanston instead of white and negro (Negroes have a different caries rate). The randomization Sutton suggests would, therefore, have introduced errors instead of eliminating them from the studies.

On the control of variability in dental caries experience, Sutton assumes no planning to have occurred. He does not realize the rather steady pattern of such variability

and the fact that it can be overcome by adequate sample size. With Muskegon, for instance, he draws attention to the fact that 12 of the samples used contained fewer than 20 children each. He does not state that there were 130 samples examined in Muskegon, all told, over a period of ten years. Seventy-nine of them contained over 100 children, and 57 of them over 200 children. The large size of the average sample in this instance is very clear evidence of a good approach to the question of variability.

It seems apparent from Sutton's comments upon examiner variability that nothing will satisfy him except the continuous employment of a very small number of examiners for the entire ten-year period of study. It seems to mean nothing to him that the studies were under continuous supervision and, if examiners changed, the new ones were oriented and standardized by the supervisor. He considers it a possible source of bias that the examiners knew in which city they were operating. In this connection, he makes the good, though difficult, suggestion that such bias might be eliminated by transporting to a common examination center children from both cities so that the residence of the child might be unknown to the examiner. He shows bias of his own, however, in refusing to credit the fact that x-rays of Newburgh and Kingston children, randomized by code number, showed results of the same order as those made by the clinical method in each city.

His most striking examples of variability are presented in connection with children from six to eight years of age, when their deciduous teeth are exfoliating, and caries in the permanent teeth is of the pit-and-fissure type which makes determination of time of onset so difficult. He neglects the much more consistent values seen among older children toward the latter part of the study period, a finding so consistently duplicated from one study to another.

It is natural that Sutton's book should have drawn comment from the principal investigators in the four fluoridation study areas which are criticized. Some of these comments are given herewith from letters to Dr. Kenneth Adamson, President of the Australian Dental Association.

#### *Grand Rapids Study*

Dr. F. A. Arnold, Jr., Director of the National Institute of Dental Research and principal investigator at Grand Rapids, writes in part as follows (*Arnold to Adamson, October 16, 1959*):

"He (Sutton) overlooks the fact that one examiner has been with the study throughout. If we used his findings, we would come up with the same general result. Also, we could call attention to the fact that two more of the four examiners used throughout the first ten years of the study started examining during the third year of the study and have participated each year since.

"He criticizes our selecting samples by school grade. If he would realize it, and probably he does, this strengthens the study. In the first place, this gives us a random sample. Also, it permitted us to examine all the children of a grade without the examiners knowing whether the child belonged to the 'continuous resident' group or not.

"Although he did not publish his material until 1959, he (apparently intentionally) overlooked the report of the tenth year of the study which appeared in 1956. As was originally planned, it was this year that we obtained 'complete' age groups of adequate size. The planning of the study and the analysis of the data were done by a group of people all of whom are more knowledgeable in this field of research than is Dr. Sutton."

#### *Newburgh Study*

Dr. David B. Ast, Director, Bureau of Dental Health of the New York State

Department of Health, makes the following comments (*Ast to Adamson, March 3, 1960*):

"Sutton criticizes the comparability of data among the four studies because in Newburgh and Kingston we used the rate based on DMF per 100 erupted permanent teeth instead of DMF per child. We explained why we used the permanent tooth population as the universe to be considered. However, in order to make our data comparable to other study data, in the reports for 1953-54 and 1954-55, the Newburgh-Kingston data were given both ways—DMF per 100 teeth, and DMF per child. What is significant and had eluded Sutton is the fact that the percentage differences in Newburgh and Kingston were almost the same for both methods used.

"Another criticism made is that base-line data were collected in Kingston a year after the Newburgh survey. I can't believe Sutton really believes this to be a valid criticism. He must be, or should be aware of the fact that caries is not an acute disease of short duration, but a slowly developing one... The base-line data in Newburgh and Kingston based on the examination of all of the school children age six to 12 in both cities were almost identical. All of the examinations were made by one examiner. Could Sutton really believe that the DMF rate of 20.8 for Kingston, and 21.0 for Newburgh could have been significantly different if both examinations were made exactly at the same time?... This type of criticism questions not the research but the professional acumen of the critic."

#### *The Evanston Study*

J. R. Blayney, Director of the Evanston Dental Caries Study, comments thus (*Blayney to Adamson, November 23, 1959*):

"Dr. Sutton... states, 'The arbitrary selection of the data which is then termed 'representative', instead of making the ultimate findings to be considered valid and reliable, would render a report based on this selective data unfit for serious consider-

ation.' We feel that this type of criticism is unworthy of the scientific stature and dignity of the University of Melbourne and would tend to imply that the rather reasonable separation of white and negro, public and parochial school children, for the purpose of comparing like with like, is an 'arbitrary selection' making the 'data unfit for serious consideration' and that the entire report hinges only on this preselected data. We have gathered no secret or concealed data...

"Dr. Sutton was concerned that the control group was not examined annually. Neither we nor our advisors could see a reason to require an examination of the control group other than at the beginning and near the close of the study. This provides the base-line from which to measure the trend of the dental caries rate during the time interval (1947-1956). Should the rate in the last examination (1956) deviate materially from that of the initial base-line period (1947), that figure could be used as a correction factor on the Evanston findings.

"It is true that a discrepancy in figures published in our paper XVI, Table I, and in paper XVIII, Table I, are at variance. This is due to the operator of the tabulating machine providing the wrong figures for the number of seven- and eight-year-old children examined. This error was discovered after manuscript XVI was in press and therefore the corrections could only be made manually in the reprints supplied to readers who requested them."

#### *The Brantford Studies*

R. M. Grainger of the University of Toronto writes (*Grainger to Adamson, December 4, 1959*):

"To be brief, the writer is correct if he is making the point that the fluoridation studies are not flawless examples of scientific literature. He is also correct if he implies that the exact amount of protection against tooth decay from fluoridation cannot be pinpointed and that its protection may not

be equal for all populations or under all circumstances. He is not correct if he implies that the slips in arithmetic, typographical errors, unsophisticated statistical analyses, minor inconsistencies between preliminary and final reports or between observer teams, which he has unearthed, throw any serious doubt on the corroborative conclusions of the water-fluoridation experiments or on the public health value of the procedure.

"Page 38, middle paragraph, re late commencement of National Health and Welfare Study and detection of caries protection for young individuals born prior to commencement of fluoridation. Despite the fact that the Department of National Health and Welfare began its control study nearly three years after fluoridation began, much worthwhile information was obtained and the effect of late commencement, if any, was to result in underestimation of the fluoride protection.

"Page 44, last paragraph, re differences in reported rates between examiners. Different examiners give characteristically higher or lower rates upon examining the same individuals due to differences in skill, training and physical condition. Thus the differences quoted are no reflection on the design of the experiment or the care taken in the work. The strength of the double examinations comes through corroboration of caries trends in Brantford over the years and not through interchangeability of data."

If Dr. Sutton had visited with an open mind such cities as Newburgh and Kingston, as this writer has done recently, he would see such obvious differences in children's mouths even on throat-stick inspection that he would stop looking for "slips in arithmetic, typographical errors, unsophisticated statistical analyses, and minor inconsistencies between preliminary and final reports or between observer teams." The same would also be true if Sutton had given a fair share of his attention to the



great mass of positive and unimpeachable findings of the various studies. Going even further, he might have included in his reasoning the great mass of supporting data on benefits of fluoridation available for studies in the endemic fluoride areas and now, increasingly, in fluoridating cities other than the four original ones.

Sutton was technically within his rights in confining his volume to destructive criticism, but the tragedy is that his work will be read by a public often unable to appreciate its defects or learn in detail the other side of the case. Worst of all the book will be used by unprincipled agitators to arouse fear in citizens' groups where fluoridation is up for community decision.

D. J. Galagan of the U.S. Public Health

Service makes remarks as follows (*Galagan to Adamson, November 27, 1959*) concerning the effect of the book upon Australian children (and this applies to some degree also to American children):

"I am sure that the report, seemingly published in good faith . . . will delay further the fluoridation of municipal water supplies in Australia. The many thousands of Australian children who will needlessly develop dental caries, and suffer unnecessarily the pains of toothache and infection, will have Dr. Sutton to thank. I think it would weigh heavily on his conscience."

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*Harvard School of Dental Medicine*  
*Boston, Massachusetts*

## LONGITUDINAL STUDIES OF CHILD HEALTH AND DEVELOPMENT

*Long-term studies of skeletal maturation, height, weight, protein and caloric intake and illness experience of 134 children emphasize individual variability at each age period but reveal individual patterns of performance.*

A number of long-term and multi-disciplinary studies of growth and development of children were begun about 1929 or the years immediately following. Reports of progress from these have appeared from time to time, but these reports have usually dealt with findings in single areas of research and for a limited age period. Some of these research projects are continuing in full or extended operations, notably one in Denver under the direction of A. Washburn and one at the Fels Institute in Ohio under L. W. Sontag. Others are operating in a modified form or have been terminated (H. C. Stuart, *Pediatrics* **24**, 701 (1959); K. Simmons and T. W. Todd, *Growth* **2**, 93 (1938); H. W. Jones, *J. Consult. Psychol.* **3**, 157, 177 (1939)).

The Longitudinal Studies of Child Health and Development, carried out by the Department of Maternal and Child Health at the Harvard School of Public Health, were started by H. C. Stuart in 1929 and conducted under his direction through

1956, at which time study of the last child enrolled had been completed. The data collected in this project include observations during pregnancy and periodic observations of growth and development from birth to 18 years of 134 children equally divided between boys and girls. Early phases of this work are described in a monograph published in 1939 after the last child had been enrolled (Stuart *et al.*, *Monograph of Soc. for Res. in Child Devel.* **4**, Serial 20, No. 1 (1939)).

Recently, a major series of reports on these longitudinal studies have appeared as a supplement to *Pediatrics* (Stuart *et al.*, *Pediatrics* **24**, 875, 886, 904, 922, 941 (1959)).

In introducing this series of papers, Stuart *et al.* state that variability in the growth and developmental progress of children is related to their health. Although growth patterns and developmental progress are determined in large measure by hereditary factors, these intrinsic factors may be modified by disease or other environmental

changes. This modification of patterns helps to separate the influences of heredity from those of environment. Nevertheless, individual differences in response to environmental factors make differentiation between "normal variations" and changes brought about by disease difficult. It is also emphasized that all of the above factors apply to the developing fetus and to the newborn infant, although our information about them is less direct. Consequently, studies of health during pregnancy are important to the understanding of growth and development throughout the whole of the growth cycle.

Since there are certain consistencies in individual growth patterns, differences between individuals deserve particular study. A number of basic concepts are inherent in the interpretation of a study of this scope. It is pointed out that there are recognizable stages in the human pattern of growth and development, each having particular health needs; thus improvement of personal health programs for children depends upon the recognition of these needs. At a given age, there is a characteristic distribution of a measurable attribute of growth and development but with differences between different groups of children. This distribution does not permit definition of normal. Moreover, individual differences in the magnitude and timing of developmental changes and in the consistency of growth patterns make individual study necessary. Knowledge of the interrelatedness between measurable attributes for individuals will reveal those attributes that are the best overall indicators of longitudinal progress.

One hundred and thirty-four children were studied. Except for six considered premature because of birth weight, these children were all full-term infants. They came from self-supporting families of Northern European stock largely of Irish extraction. Inclusion in the study group depended upon attendance at the prenatal clinic of the Boston Lying-In Hospital and willingness to participate.

The various papers in the present monograph report the patterns of skeletal development in the hand, of growth in height and weight, caloric and protein intakes, and patterns of illness experience. Data concerning interrelationships between these measurable attributes is planned for publication in the near future.

Skeletal development was assessed by comparing radiographs of the hand with the standards of W. W. Greulich and S. I. Pyle (*Radiographic Atlas of Skeletal Development of the Hand and Wrist*, Stanford University Press (1950); second edition (1959)). The mean values for the group as a whole were slightly faster than the standards for infancy, slightly slower during childhood, and slightly faster during adolescence. These deviations were representative of the group but were not statistically significant.

Some individual patterns (determined by plotting skeletal age (hand) versus chronological age) were consistently faster or slower than the standards, while others were variable. By assessing the relative rate of skeletal development the children were divided into those who had a consistent pattern during the whole 18 years of the study, those who had differing rates at two age periods, those with differing rates at three age periods, and finally those whose pattern was irregular. By assessing individual patterns as slow, moderate, or fast, 13 patterns were found out of a possible 21.

Patterns were classified as fast or slow if they changed more than one standard deviation during the age period under consideration. Twenty-seven children had a moderate rate of skeletal maturation during the whole of the growth period. Fifteen children who grew more rapidly during the second half of the growth period had had a moderate rate initially. Fourteen children had an initial slow rate and a subsequent fast rate. Eighteen children were classified as irregular. To classify the results somewhat differently it was felt that 35 children had consistent patterns, another 35 speeded

up their rate of skeletal maturation in later childhood and adolescence, 18 had irregular patterns and the rest fell into a number of different groups.

The results are presented graphically in a manner emphasizing individual variation. The great mass of observations is at first bewildering, but the reader soon grasps the system of classification. One of the more interesting findings was that a majority of the twins had very similar patterns of skeletal maturation even if they were not identical twins. Other children had very similar patterns suggesting the possibility of genetic similarity in individuals who were not related.

Accelerated patterns of skeletal maturation were seen in children who at one year of age were either delayed or advanced. It is interesting that this acceleration was greater in the children considered advanced in bone age at one year than it was in those whose bone age was relatively slow at one year. The similarities between sexes were impressive using standards which were adjusted for the more advanced bone age of girls.

It is concluded, therefore, that there is great variety in the rate of skeletal maturation in normal children. The great majority of children have patterns of bone maturation which fall into a few general categories. The most common change in pattern is acceleration between childhood and adolescence.

Data on height and weight are reported in yearly averages and ranges for the two sexes separately. The 18 years under study were divided into three periods of six years each; the total growth increment was calculated for each period and its relative magnitude assessed according to the group as a whole. The individuals falling in the extreme quartiles of the distribution were designated as slow or rapid during the six-year period, while those within the median 50 per cent were considered moderate in growth rate.

The 134 individuals in the study fell into

five main groups. Those with rapid growth during the first six years of life tended to mature early, while those with slow growth during the first six years of life had a later pre-adolescent growth spurt. For those with moderate growth in the pre-school years, the time of the pre-adolescent growth spurt was variable. The correlation of growth before six years with final height at 18 years was also noted.

Similar patterns were seen for both height and weight increments and emphasize the range of variation. A number of figures in this article showing the patterns of individual growth curves against the background of the group as a whole demonstrate the range of variation better than any statistical analysis.

The caloric and protein intakes were estimated by "the Burke dietary history method." Since 66 per cent of the infants were breast-fed for varying periods during the first year of life, no data were reported for infants under the age of one year. Yearly averages from one to 18 years are given for calories and protein. There was a great deal of variation in the data, with one child having twice the intake of calories and protein as another child in the same age and sex group. In all ages the males had greater intakes than the females, except for the protein intake under six years of age. The averages were slightly higher than those of the Recommended Dietary Allowances of the National Research Council (*NAS-NRC Publication 589, Washington, D. C. (1958)*), except for the caloric intake of the girls. In comparison with other recent studies of caloric and protein intakes of American and British children, reasonably close agreement was found.

The longitudinal cumulative intakes in the various age periods to 18 years were divided into high, medium and low categories and many different patterns and relative changes with respect to age were encountered. There were no clear-cut patterns to represent the dietary habits of large numbers

of children. Although the authors made no effort to relate caloric to protein intake, a few calculations from the data suggest that the great majority of the children of all ages had protein intakes representing about 12 to 14 per cent of calories.

In dealing with the illness experience of 134 children, each followed for almost 18 years, a quantitative assessment was extremely difficult. An elaborate classification of illnesses as to number, severity and duration was adopted and the available historical data were all reviewed by one individual. Numerical scores for illness experience were evolved which ranged from 1 to 25 for single illnesses.

As might be expected, the boys had a greater number of illnesses than did the girls although the scores for severity of illness were only slightly higher in the males. Both the number of illnesses and the severity scores were highest in the pre-school ages

and lowest in the adolescents. In order to evaluate possible patterns of illness experience within the group, classifications were made based on score and frequency of illnesses. This resulted in the characterization of 14 separate patterns.

This monograph represents only a small portion of the data collected in the long-term study of these 134 children. The techniques employed to develop objective means of assessing long-term patterns of growth, development, dietary intake and illness experience are novel and constitute a valuable contribution to methodology. The presentation of such a mass of information without any attempt at intercorrelation of the various parameters measured is extremely tantalizing to the reader. It is hoped that the relationships which might exist between illness experience, growth, maturation and nutrition have been studied as extensively as the basic observations.

### HOOKWORM INFESTATION AND ABSORPTION

*Severe hookworm infestation has no effect on calorie absorption, but produces a slight reduction in the absorption of nitrogen; mild infestation has no effect on either calorie or nitrogen absorption.*

Individuals who are heavily infested with hookworms are usually anemic. The anemia is frequently associated with the loss of blood from the intestinal tract. Until recently it was believed that the intestinal hemorrhages with the accompanying loss of blood were entirely responsible for the poor nutritional condition of the hookworm-infested patients (*Nutrition Reviews* 6, 181 (1948)).

S. J. Darke (*Brit. J. Nutrition* 13, 278 (1959)) has shown that natives in Tanganyika who have a high count of hookworm eggs in their stools show a reduction in the absorption of dietary nitrogen. She was led to this study by the observation that the stools from anemic patients who were free from or only mildly infested with hookworms had  $54.6 \pm 2.7$  mg. of nitrogen

per gram of dry material, whereas the stools from patients with heavy hookworm infestation had  $61.8 \pm 1.7$  mg. There was no difference in the energy contents of the stool. On the basis of these observations, the following studies were carried out.

Eight subjects for the study were from Kampala while one was a patient in the metabolic ward of the East African Institute for Medical Research. The subjects included two children, one of whom was six years old and weighed 15 kg. while the other was 12 years old and weighed 20 kg. The other subjects were adults whose ages were unknown.

All patients had occult blood in their stools and had large numbers of hookworms in their intestines. The stools passed during the 36 hours following a deworming dose of tetrachlorethylene and magnesium sulfate

contained, by actual isolation, from 416 to 2839 worms. The low value was for the six-year-old child, however, while most of the subjects passed more than 1000 worms. These counts were obviously minimal values. All subjects were severely anemic as shown by hemoglobin levels ranging from 30 to 46 per cent.

Balance studies were carried out on each subject while on the hospital diet. The food consisted of maize porridge made with water and a little milk, bread, cooked bananas, groundnut soup and either rice soup plus meat (three times a week) or sweet potatoes plus beans (four times a week). Unfortunately, the length of the balance periods was not given.

The percentage of calories absorbed as determined by bomb calorimetry of the diet and stool, ranged from 76.5 to 90.5 per cent with a mean of  $86.1 \pm 1.3$ . The intakes ranged from 1659 to 2362 calories except for the six-year-old, who received 800 calories. A week or more after deworming, the balance studies were repeated on three of the subjects and were also carried out on two other natives who were worm-free. In the absence of worms, the subjects absorbed an average of 90.0 per cent of the dietary calories. There was no significant difference in the absorption of calories between the group heavily infested with worms and the one that was worm-free.

The absorption of nitrogen in the patients heavily infested with hookworms ranged from 41.1 to 78.4 per cent, with an average of 62.5 (S.E. = 3.1). The absorption of nitrogen in the worm-free patients ranged from 67.7 to 81.6 per cent with a mean of 73.3 (S.E. = 1.9). The difference in the percentage of absorption of nitrogen (10.8) between the two groups was significant. These absorption values were secured with diets which provided a nitrogen intake for the individual subjects ranging from 5.2 to 10.9 g. per day.

Darke (*loc. cit.*) emphasized the fact that the improvement in nitrogen absorption

following deworming occurred before there was any change in the anemia. On this basis, the reduction in nitrogen absorption may be considered a primary effect of the heavy hookworm infestation.

In a similar study carried out among other natives in Tanganyika, E. G. Holmes and S. J. Darke (*Brit. J. Nutrition* **13**, 266 (1959)) found that the absorption of both calories and nitrogen was normal in patients with mild hookworm infestation. The number of hookworms isolated from the stools of some of these adult patients was usually less than 200. None of these subjects was anemic and they were presumably in better nutritional condition than the heavily infested natives studied earlier.

Eleven observations (length of balance periods and numbers of subjects not stated) were made on the absorption of calories and nitrogen before and after the subjects were dewormed. Before deworming the subjects absorbed  $73.5 \pm 2.0$  per cent of the dietary calories while afterwards they absorbed  $70.9 \pm 2.2$ . Comparable values for nitrogen were  $88.9 \pm 0.7$  per cent before and  $87.6 \pm 0.8$  afterwards. The caloric intake of these subjects ranged from 2256 to 3795 calories per day, while the nitrogen intake ranged from 14.1 to 23.8 g.

The African natives who showed a very high infestation of hookworms were primarily migrant laborers who were poorly paid and consumed a poor diet. In the same area, however, there are other natives whose diet is fairly good, presumably because of an improved economic status. Although the latter are also exposed to hookworm infestation, they harbor a relatively small number of these parasites in their intestines. Nevertheless, there is little concrete evidence that the good diet affords protection against heavy parasitic infestation. Darke (*loc. cit.*) indicates that her clinical experience suggests that "it is rare to find heavy hookworm loads with severe anemia in persons whose diet is not or has not been recently grossly deficient in protein."

## ASSESSMENT OF WEIGHT REDUCTION

*Evaluation of reduction diets is difficult since criteria for evaluation are variable. A mathematical formula has been devised to compare data of diverse origin.*

The common problem of obesity often frustrates both patient and physician. Simple restriction of calories with maintenance of an adequate diet should solve the problem. However, this program fails so often that fads, innovations and even dangerous practices flourish. One of the important factors accounting for failure is a lack of understanding of a precise estimate of normal weight, of the rate at which weight should be lost, and of the number of calories which should be eaten to achieve this hypothetical weight in a given period of time.

Much uncertainty in the minds of physicians has been created by the disparities found in published reports regarding therapy of obesity. A. R. Feinstein (*J. Chronic Diseases* 10, 439 (1959)) has discussed these difficulties and has suggested a means whereby a uniform measure of obesity and of the results of treatment could be applied to all investigative studies.

The first problem was to assess the degree of obesity. This has been done in many ways including measurement of the thickness of a fat fold (*Nutrition Reviews* 9, 265 (1951)), measurement of the specific gravity of an individual (*Ibid.* 10, 247 (1952)), and the use of tracer substances. One of the oldest techniques has been the use of reference tables based upon age, sex, height, and average weight of a large group of individuals. One such tabulation which came into popular use has been revised slightly throughout the years and continues to be a reasonably valid reference for persons in the United States (*Medico-actuarial Mortality Investigations, Assoc. Life Insur. Med. Dir. and Actuarial Soc. America, vol. one* (1912)). Accordingly, Feinstein accepted the tabular value as representative of the desirable weight for a given patient. He did,

however, take into consideration such subjective factors as appearance, general build, and the distribution of fatty tissues.

Dietary success or failure depends upon achievement of a predetermined desirable weight selected on the basis of the above-mentioned criteria. Achievement in dietary treatment of obesity must be considered as occurring in two phases; the first, a period of active dieting when weight is lost fairly rapidly, and the second, a more prolonged period during which time the patient maintains the more desirable weight achieved. Loss of weight must occur without undue physiologic or psychologic derangements.

The author discusses a number of methods for measuring achievement of weight reduction. The simplest is a mere expression of the initial weight in pounds and the number of pounds lost after treatment. A further refinement is division of the weight lost by the duration of time. This has the disadvantage, however, of giving undue credit to extremely obese patients who might lose rapidly for a short period of time. Conversely, a less obese patient who lost weight more slowly and physiologically would seem, statistically, to be a failure. Another index of performance was described by N. Jolliffe and E. Alpert (*Med. Clin. North America* 37, 733 (1953)) which consisted of the ratio of actual loss to the anticipated loss times 100. This formula had the advantage of demonstrating the degree with which a patient adhered to his diet, but it did not assess the amount or relative worth of the weight loss.

Still another popular expression of the degree of obesity is in terms of per cent above desirable weight and per cent of weight loss. Some years ago this technique was introduced in a tabular form which



indicated in arbitrary figures whether a patient's dietary regimen had been successful or unsuccessful based upon the per cent weight loss achieved (M. Trulson, E. D. Walsh and E. K. Caso, *J. Am. Dietet. Assn.* **23**, 941 (1947)). The major defect in this system was that it did not provide for an expression of the effect of surplus weight. In effect, it avoided the problem of selecting a target weight and gave undue credit to massively obese patients.

Feinstein, with an admirable penchant for mathematical formulas, established a relatively simple and logical formula which took into account each of the factors mentioned above. These included weight lost, surplus weight, target weight, initial weight, final weight (lowest achieved during dieting) and duration of time. He established what he termed a reduction coefficient (R.C.) by which he could estimate the degree of obesity of any patient. This consisted of the initial weight of the patient divided by the product of the surplus weight and the target weight, times 100. Obviously both the surplus and target weights had to be derived from the estimated figure for the patient's theoretical ideal, but practical use revealed that the variation in R.C. produced by arbitrary choice of target weight was not excessive.

The characteristics of the reduction coefficient (R.C.) are as follows: (1) The higher the initial weight of the patient, the less will be the value for the available range of variation of R.C. (2) If patients who are less than 20 per cent obese are omitted, the complete range of R.C. is from 1.3 to 5.5. For example, a patient 30 per cent overweight would have an R.C. value of less than 4.0 and a patient 40 per cent overweight would have an R.C. value of less than 3.0. (3) For the same initial weight, R.C. becomes larger as the target weight is increased. In other words, R.C. represents a reciprocal of the excess weight. Similarly he used a reduction index (R.I.) which can be obtained by multiplying R.C. by the

weight loss in pounds. These formulas were then applied to clinical experience.

A series of 106 patients who were treated for obesity during a dietary study were evaluated by the above-mentioned criteria. The average weight of the group was 224 pounds and the average surplus weight was 83 pounds. By applying the criteria of success or failure as described by Trulson, Walsh and Caso (*loc. cit.*) it was found that a reduction index (R.I.) value of greater than 60 implied a fair degree of success while an R.I. value of less than 60 implied relative failure. Of his 106 patients, Feinstein found that the average R.I. value was 66 and that 47 per cent of the series had a value greater than 60. He then proceeded to apply this type of statistical evaluation to the data supplied by 13 other publications in order to compare the validity of his technique. The results expressed in tabular form would suggest an easy method for comparing the relative effectiveness of the various dietary regimens used in these 13 series of patients. At a glance, one can compare R.I. values of different groups of patients.

Such comparisons are most desirable since many diets and dietary adjuncts have been advocated by various investigators but the results of these therapeutic attempts have not been expressed in a common language which can be readily grasped. Perhaps this has contributed to the lack of confidence which many nutritionists place in the published reports of other workers not well known to them.

The formulas proposed by Feinstein seem to be simple, logical and capable of providing a universal basis for comparison of data collected by any group treating obese patients by dietary methods. Whether these formulas will prove to be universally successful, or will indeed be accepted at all, remains to be seen, but the author has applied logic and common sense in an attempt to find a common denominator which might help to solve a confusing problem.

## AMINO ACID AVAILABILITY IN MAN

*Beef protein scores highest in terms of nitrogen and amino acid availability when compared to milk and egg, cottage cheese, pork, milk alone, peanut butter and the control whole-egg protein.*

In the third article of a series on the biological availability to human subjects of essential amino acids, J. H. Watts, C. H. Allen and L. K. Booker (*J. Am. Dietet. Assn.* **36**, 42 (1960)) have measured the availability of the eight amino acids essential to man in diets containing whole egg and beef muscle. Previous reports from the same laboratory have described measurements of the availability to man of the eight essential amino acids and of cystine and tyrosine in diets containing whole egg, pork muscle, peanut butter, egg and milk, milk alone and cottage cheese (*J. Nutrition* **67**, 483, 497 (1959); *Nutrition Reviews* **17**, 268 (1959)).

The three women and two men who were subjects of the present study were between 22 and 27 years of age and ranged in body weight from 44 to 74 kg. Four were graduate students and one a research assistant. Normal class and work schedules were followed by them during the feeding experiments. Experimental methods and design were similar to those of the earlier studies mentioned above, except that no attempt was made to correct for fecal amino acids of both endogenous and bacterial origins by feeding the low-protein basal diet in the absence of a protein test food. The low-protein regimen was omitted, since in the previous studies the fecal amino acids during feeding of the low-protein diet exceeded those measured during protein feeding. This observation led the authors to conclude that the amount of metabolic amino acids in feces varies with the type of diet and, therefore, that the inclusion of a low-protein regimen in the experimental design does not provide suitable estimates for endogenous amino acids or those synthesized in the intestine.

During a preliminary experimental period

of ten days, it was determined that 0.8 g. of nitrogen (from whole-egg protein) per kg. body weight per day and 48 calories per kg. per day were required to maintain slightly positive nitrogen balance and constant body weight. Diets for each subject were held isonitrogenous and isocaloric (by sucrose supplements) during each of two subsequent ten-day experimental periods. The first four days of each period were set aside for adjustment and no urinary or fecal collections made.

The basal diet contained between 4.5 and 5.0 g. protein per day. A portion of the egg or beef protein added in the experimental periods was eaten at each of three meals. The experimental diets contained from 157 to 250 g. of beef and from 260 to 420 g. of whole egg. Total nitrogens (Kjeldahl) were measured in diets, feces and urines, and the contents of individual amino acids (microbiological assay) were measured for diets and feces. The per cent availabilities of nitrogen and of each of the essential amino acids were computed by multiplying by 100 the quotient of dietary intake less fecal excretion divided by dietary intake.

During the egg-feeding and the beef-feeding periods, mean daily nitrogen intakes were 7.01 and 7.06 g., respectively; mean daily urinary nitrogen contents were 4.24 and 5.18 g.; mean daily fecal nitrogen contents were 0.93 and 0.74 g.; mean daily nitrogen balances were +1.87 and +1.23 g.; and mean calculated percentages of available nitrogen were 86.9 and 89.6.

The dietary intake of each essential amino acid approximated or exceeded the safe intakes defined by W. C. Rose (*Current Research in the Science of Nutrition. The Nutrition Foundation* (1955)), provided an allowance was made for the methionine sparing effect of cystine. The mean calcu-

lated percentage availabilities of individual essential amino acids during egg-feeding and beef-feeding periods, respectively, were: isoleucine, 90.3 and 92.5; leucine, 90.9 and 92.8; lysine, 89.6 and 92.8; methionine, 91.4 and 90.4; phenylalanine, 90.3 and 90.2; threonine, 89.0 and 89.3; tryptophan (mean value based on three subjects only), 76.0 and 79.5; and valine, 89.8 and 90.6.

The authors interpret their data as indicating an equality of egg and beef protein with regard to the availability of essential amino acids. They point out that their data in man, while qualitatively similar to other data collected in animal investigations, do not bear out the superiority of egg protein over beef as reported for isoleucine by P. D. Deshpande, A. E. Harper, M. Collins and C. A. Elvehjem (*Arch. Biochem.* **67**, 341 (1957)), and for lysine by B. T. Guthneck, B. A. Bennett and B. S. Schweigert (*J. Nutrition* **49**, 289 (1953)) and by J. D. Gupta, A. M. Dakroury, Harper and Elvehjem (*Ibid.* **64**, 259 (1958)).

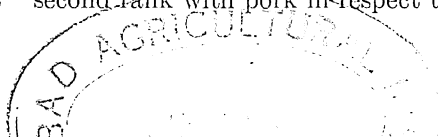
Comparisons of the percentage availabilities of nitrogen and individual amino acids in egg and beef protein in this study with those for whole egg, pork muscle, peanut butter, egg and milk, milk alone and cottage cheese, calculated from data obtained in the previous studies (see above), are complicated by the fact that different subjects were used. Only male subjects were used in the preceding studies while the sexes are not indicated in the present report. Moreover, a dietary protein intake of 1.0 g. per kg. body weight per day was deemed essential for maintenance of nitrogen balance in the previous studies, whereas 0.8 g. per kg. per day was selected in the present work.

Nevertheless, if taken at face value, the mean calculated percentages of available nitrogen in previously tested proteins were, respectively: whole egg (study I), 89.0, whole egg (study II), 88.4 (compared with 86.9 per cent in the present study); pork muscle, 90.0 (compared with 89.6 per cent

for beef); peanut butter, 86.5; egg and milk, 89.6; milk alone, 83.3; and cottage cheese, 89.4. Except for the value of milk alone, these values are not strikingly different from one another. Considering the wide range of individual values included in the mean and the small number of subjects involved, the significance of small differences among the various proteins is very slight.

If the whole-egg protein experiment in each study is considered as a built-in control, and if the mean calculated percentage availability for the respective whole-egg protein experiment is subtracted algebraically from the mean calculated percentage availability for the experimental period during which another protein was fed, a value is obtained for the difference in mean calculated percentage availability of nitrogen between the experimental protein and the whole-egg control. The authors' data, viewed in this way, afford the following rank order for the differences in mean calculated percentage availabilities for nitrogen: beef (+2.7), milk and egg (+1.2), cottage cheese (+1.0), pork muscle (+1.0), peanut butter (-2.5) and milk alone (three subjects only) (-5.1).

When the same consideration is given the data on amino acid availability, rank orders of the six proteins tested may be established with respect to the difference in mean calculated percentage availability of a given amino acid between the experimental protein and its whole-egg control. The ranks (one to six) of each protein with respect to each amino acid may then be summed to obtain a score (equal weight being given each amino acid). When such scores are computed, beef protein has the low (best) score of 13.5; milk and egg, 17.5; cottage cheese, 24.0; pork muscle, 25.0; milk alone, 42.0; and peanut butter, high (worst) score, 46.0. Beef protein ranks first in the availability of isoleucine, leucine, lysine, tryptophan and valine, third for methionine and threonine, and is tied for second rank with pork in respect to phenyl-



alanine. Except for methionine and phenylalanine, the essential amino acid availabilities in beef were greater than in the whole-egg control. Except for the reversed order of milk and peanut butter, the same rank order is obtained from considerations of nitrogen availability alone.

Such manipulations of the authors' data only tend to bring out more clearly what information may be gleaned from the experimental efforts the data represent. Such manipulations do not lessen, however, the underlying weakness of an experimental design which assumes no bacterial synthesis or endogenous release of amino acids (*Nutrition Reviews* 17, 268 (1959)). Nor does it lessen the weakness of a calculation of availability based on the difference between dietary intake and fecal excretion.

Inevitably a finite quantity of intake is lost in the feeding process, and equally inevitably a finite quantity of feces is not recovered. When fecal output is subtracted from intake the two losses are additive, resulting in a falsely high value for availability. Demarcation of short fecal collections by carmine markers is subject to large error, adding greatly to intra- and interindividual variation of data based on isolated short collection periods.

In general, the present methodology used for studying amino acid availability in adult man is of questionable adequacy, no matter how carefully performed. This inadequacy involves most amino acid research in man and thus study by new, more ingenious and reliable techniques is essential.

#### TREATMENT OF HYPERCHOLESTEROLEMIC PATIENTS WITH NIACIN, SITOSTEROL AND SAFFLOWER OIL

*Although large doses of niacin or safflower oil or sitosterol decreased serum cholesterol in man, administration of sitosterol and niacin together was more effective.*

There is little doubt that coronary disease occurs more frequently in hypercholesterolemic patients than it does in normal persons (*Nutrition Reviews* 7, 155 (1949)), but the evidence for a cause-and-effect relationship is inadequate. The answer will have to await the development of a technique whereby the concentration of serum cholesterol can be regulated with ease so that clinicians will be able to evaluate the effect of lower levels of cholesterol upon the incidence and prognosis of coronary disease.

Of the many methods available for lowering the concentration of serum cholesterol, one has been substitution of unsaturated fats for saturated fats. Other methods include the administration of niacin, vanadium, estrogens, sitosterol or safflower oil (*Nutrition Reviews* 17, 231, 291, 342 (1959)).

From a preliminary study of 54 hypercholesterolemic patients, K. G. Berge, R. W. P. Achor, N. W. Barker and M. H.

Power (*Am. Heart J.* 58, 849 (1959)) concluded that niacin in large doses could suppress the concentration of serum cholesterol for prolonged periods of time. They then attempted to assess the effectiveness of this substance in comparison with two other agents, safflower oil and sitosterol. Ten hypercholesterolemic patients were treated with 5.3 g. of niacin per day for nine to 17 months and given a placebo for three months. Then for three months they were given emulsion of safflower oil (containing 46 per cent linoleic acid glyceride), one ounce three times daily, or 20 per cent suspension of beta- and dihydro-beta-sitosterols, one ounce before each meal. Nine of the subjects received both sitosterol and niacin (4.3 g. per day) simultaneously. Determinations were made of the serum cholesterol at intervals of two to four weeks during the study.

The results showed an effect from each

of the medications despite the continuance of a usual diet. During the period of placebo administration, the average concentration of serum cholesterol was 341 mg. per 100 ml. (range 277 to 522). The average changes for the group, expressed as per cent decrease from 341, were as follows: niacin, 24 per cent; safflower oil, 6 per cent; and sitosterol, 13 per cent. There was little correlation between the response of a given patient to one agent or another. The average weight of the patients varied slightly as follows: placebo, 146 pounds; niacin, 144 pounds; safflower oil, 150 pounds; and sitosterol, 147 pounds. Safflower oil added approximately 540 calories to the daily diet.

Combined therapy of nine patients with both sitosterol and niacin (average dose 4.3 g. per day) resulted in a mean decrease in cholesterol level of 25 per cent when compared with the level during placebo administration (346 mg. per 100 ml.). On the other hand, administration of 4.3 g. of niacin alone per day to these patients caused only a 17 per cent decrease in cholesterol level, and sitosterol alone a 12 per cent decrease.

Side reactions which accompanied administration of niacin were flushing, itching and gastrointestinal distress. Occasional abnormalities were found in the results of the cephalin-cholesterol flocculation test and in the concentration of alkaline phosphatase and glutamic-oxalacetic transaminase in the serum. There was no evidence of clinical hepatic disease. There was also some impairment of the carbohydrate tolerance test. All of these abnormalities were reversed as soon as niacin was dis-

continued. Neither sitosterol nor safflower oil caused any discomfort other than unpalatability and a tendency to cause loose stools.

The authors discussed possible mechanisms whereby these agents might reduce the concentration of cholesterol in the serum. Presumably sitosterol inhibits absorption of cholesterol from the gut. Unsaturated oils are most effective if substituted for saturated fats, but, aside from the factor of essential fatty acids, the reason is obscure. Niacin is effective but its amide is not, even though it also is an active vitamin. The cutaneous flushing and itching seem to be inseparable from the cholesterol lowering effect. Explanations for this have ranged from an oxidative enzymatic process (J. M. Merrill, *Circulation Res.* 6, 482 (1958)) to enhancement of the metabolic rate (R. Altschul and A. Hoffer, *Arch. Biochem. Biophys.* 73, 420 (1958)). Niacin induces urinary excretion of nicotinuric acid but niacinamide does not. Whatever the mechanism, it must be a pharmacologic one since the necessary dose is so great.

This report describes an experimental method whereby hypercholesterolemia can be reduced by 25 per cent without dietary change or undue risk. This must be considered an investigational form of therapy, and should not be adopted as a routine measure until more experience has been gained. The possibility of late toxic effects needs further study. Finally, it will require critical evaluation to determine whether artificial lowering of the concentrations of cholesterol in the serum is actually of any value to the patient.

## NITRATES OR NITRITES AND ABORTION IN CATTLE

*Nitrates or nitrites in the diets of pregnant dairy cattle produced non-infectious abortion similar to that observed in cattle on unimproved marshland pasture. Sodium nitrite was more toxic than potassium nitrate.*

The problem of non-infectious abortion in dairy cattle has concerned veterinarians and

dairymen for many years. In fact, one form of this type of abortion has been reported

among cattle on unimproved marshland pasture in Wisconsin for the past 50 years. Early work from the Wisconsin Agricultural Experiment Station indicated that nitrates or nitrites present in rather high levels in certain species of weeds were probably responsible for this abortion syndrome.

J. Simon *et al.* (*J. Am. Vet. Med. Assn.* **135**, 311 (1959)) have recently reported on experiments in which they attempted to correlate the quantity of nitrate or nitrite in the feed with the development of an abortion in pregnant dairy heifers. Earlier observations of oat-hay poisoning (W. B. Bradley, H. F. Eppson and O. A. Beath, *Wyoming Agric. Exper. Sta. Bull.* **241** (1940)) indicated that the lethal dose of potassium nitrate for cattle was 25 g. per 100 pounds of body weight. E. I. Whitehead and A. L. Moxon reported (*South Dakota Agric. Exper. Sta. Bull.* **424**, 1 (1952)) that excessive nitrate in the diet would result in abortion.

Simon *et al.* postulated that a continuous intake of nitrate or nitrite in sufficient quantity would produce a methemoglobinemia and that the resulting anoxemia in the fetal calves would cause death and subsequent abortion. They set up experiments in which pregnant heifers were given varying levels of potassium nitrate or sodium nitrite at regular intervals in an effort to ascertain whether the resulting syndrome of abortion was identical with that occurring on the marshland pastures.

Ten grams of sodium nitrite given twice daily for three weeks produced methemoglobin levels as high as 12 per cent of the hemoglobin after one week. At the end of two weeks this had risen to 18 per cent, but did not result in abortion. One cow produced a normal calf shortly after the nitrite was discontinued, but the fetal membranes showed lesions similar to those reported for marshland abortions. Interestingly enough, 40 g. of potassium nitrate fed twice daily for three weeks gave no signs of illness or abortion and no measurable methemoglobin in the blood.

In a more critical experiment, Simon *et al.* fed potassium nitrate by capsule twice daily at levels ranging from 10 to 20 g. per 100 pounds of live weight. Those animals given 20 g. per 100 pounds of live weight died following the second dose, having received a total of 140 g. of potassium nitrate. All of the animals receiving the intermediate level, a total of 100 g. of potassium nitrate per day, aborted and the lesions of the fetal membranes resembled those seen in the naturally occurring abortions on marshland pasture. Those animals receiving 10 g. per 100 pounds of live weight did not abort, although they did develop high levels of methemoglobin in their blood.

These very interesting studies emphasize that animals on poor grass hay or pasture may be far more susceptible to nitrate or nitrite poisoning than animals on diets high in carbohydrate concentrates. Certainly this work shows conclusively that nitrates or nitrites will cause severe methemoglobinemia and that one of the results in pregnant cattle may be abortion associated with anoxemia.

It is also evident from a consideration of this work that the methemoglobin levels of blood do not consistently reflect the level of nitrate or nitrite in the diet. Furthermore there was a wide variation between different animals on the same nitrate level, suggesting that methemoglobin in the blood is a poor measure of nitrate toxicity. Apparently individual variation, diet composition, sampling technique, time from blood sampling to analysis and method of analysis all influence methemoglobin values.

Simon *et al.* suggest that some better technique of measuring the effect of nitrate and nitrite is required and propose the analysis of nitrate in the blood as a method for determining the reaction of cattle to nitrate in the feed.

While the work of these authors has established the close relationship between nitrate or nitrite in non-infectious abortion



in dairy cattle, the limited number of animals, the individual variation and the wide spread in nitrate levels used prevented the determination of levels at which this syndrome will develop. More particularly, they have not indicated the levels at which nitrite, a more toxic ingredient than ni-

trate, will result in abortion nor what is the likely relationship between nitrite and nitrate under naturally occurring pasture conditions. These questions are of particular importance to dairymen and cattlemen in areas where high nitrate levels in pasture forage are a problem.

## BLOOD LOSS AND PLASMA CHOLESTEROL LEVELS

*The removal of blood samples from rats may be responsible for increases in plasma cholesterol levels.*

In many projects using small laboratory animals, the effect of diet on plasma cholesterol levels is studied by taking samples of blood at frequent intervals.

In the course of such a project, I. Coleman and J. M. R. Beveridge (*Nature* **184**, 1041 (1959)) found that some of the increases observed in the cholesterol levels with a high-fat diet in one rat experiment were not reproduced when the experiment was repeated. The only difference in the conditions of the two experiments that might provide an explanation for this was in the frequency of the blood samples and their size.

In the first experiment, samples corresponding to 2 per cent of the blood volume of the rats were taken on alternate days for the first eight days, and then at weekly intervals for a month. In the second experiment lasting two weeks, samples corresponding to 0.5–1.0 per cent of the blood volumes were taken on the first and last day only, and there was no change in cholesterol level in the rats receiving the high-fat diet.

Two further trials were then set up to measure the response of rats to two experimental diets under different degrees of bleeding stress. In one diet, just under 60 per cent of the calories were calculated to come from corn oil, while the second diet was essentially fat-free. Each trial lasted for six days and the rats were bled on alternate days.

In the first of the two trials, using twenty

animals for each diet, the initial level of plasma cholesterol was approximately 72 mg. per 100 ml. Six days later after three blood samplings (each of which were equivalent to 1.0–1.5 per cent of blood volume), the values for the high-fat diet had increased significantly by 31 per cent while those for the fat-free diet had increased by only 10 per cent (a nonsignificant difference). In the second trial, in which 15 animals were allotted to each diet, the mean initial level of plasma cholesterol was 67 mg. per 100 ml. The conditions were the same as for the first trial except that the samplings corresponded to 2.5–4.0 per cent of blood volume on each occasion. After six days, the values on the high-fat diet had increased by 122 per cent, and on the fat-free diet by 59 per cent.

With respect to experimental design, it was unfortunate that the size of blood sample was not used as a variable within a test. Nevertheless the results do strongly suggest that the taking of ordinary blood samples can itself be a factor influencing plasma cholesterol levels in experiments with rats. Work has been reported previously by J. J. Spitzer (*J. Lab. Clin. Med.* **46**, 461 (1955)) confirming the older observations that hemorrhage could result in lipemia in rabbits, but showing that daily blood losses of the order of 10 to 15 per cent of blood volume were needed in order to observe the phenomenon. The suggestion from the present work is that very much

smaller losses can precipitate cholesterolemia in rats.

The conclusion seems to be that experimentalists should take care to equalize the blood losses of experimental animals receiving different treatments; and also that they should record in their publication what

degree of bleeding stress has been imposed. As is pointed out by Coleman and Beveridge, some of the conflicting reports about the response of the plasma cholesterol level to high-fat diets may be explained by differences in bleeding frequency and sample size in different laboratories.

## GROWTH RATE AND LYSINE

*Evidence is offered that differences in rates of growth of chicks do not, per se, necessitate different dietary levels of lysine (expressed as a percentage of the diet) for optimum growth.*

One of the explanations for the occurrence of suboptimal growth with highly purified diets apparently containing all nutrients, including amino acids, is that the rate of growth of an animal might influence its percentage requirement for essential amino acids. However, P. Griminger (*Doctoral Thesis, University of Illinois, Urbana (1955)*) found no significant differences in requirements for methionine, tryptophan and lysine between fast- and slow-growing chicks.

On the other hand, H. M. Edwards *et al.* (*Poultry Sci.* **35**, 385 (1956)) observed that, to obtain optimum growth with diets containing wheat gluten or sesame meal, a different percentage level of lysine was required for each diet, *i.e.*, the chicks receiving the sesame meal had a higher requirement for lysine. Since these chicks also grew at a much greater rate than those receiving the wheat gluten diet, the authors interpreted this to mean that the lysine requirement of the chick is related to the rate of growth.

Because the findings of Edwards and co-workers seemed to be at variance with his own, Griminger, working with H. M. Scott (*J. Nutrition* **68**, 429 (1959)), has carried out a number of experiments to determine whether the rate of growth actually does, *per se*, influence the lysine requirement (expressed as a percentage of the diet) of the growing chick. Lysine was used as the limiting amino acid since it is concentrated

to the greatest degree in muscle and organ tissue. Thus it would appear likely that the requirement for this amino acid is influenced by rate of growth.

The requirement for lysine in growing chicks was determined under four different conditions: (1) with chicks from a single population but selected for fast and slow growth, (2) with and without a non-nutritive growth depressant (saponin), (3) with chicks from different breeds growing at different rates, and (4) with and without inoculation of the chicks with bronchitis virus.

In the first experiment, optimum gains, weights and growth rates for both groups were obtained at the 1.03 per cent level of lysine. However, analysis of the growth rates revealed that the terms "fast"- and "slow-growing" for the two groups were only relative. The growth results, when broken down into growth groups at two weeks of age, showed a clear tendency for the smaller chicks to add more weight on a percentage basis than the larger chicks. Consequently, there should be a smaller maintenance requirement and it was found that the gain to feed ratios were more efficient for the smaller chicks. Statistical analysis revealed that final growth data were significantly related to a particular growth group or to the lysine level, but that no significant interaction exists between these two factors.

Although administration of saponin reduced gains and growth rates to approximately two-thirds of normal, it did not alter the percentage level of lysine at which optimum growth took place. Similarly, the chicks from a highly inbred line of white Leghorns registered optimum gains and growth rates (within their genetic capabilities) at the same level of lysine (0.93 per cent). Optimum gain to feed ratios were also obtained at this level of lysine. Both the saponin and Leghorn groups had lower feed efficiency values than the control group.

In the last experiment, using the bronchitis virus as a growth depressant, optimum growth was obtained at the 1.11 per cent level of lysine for both the injected and control groups. Growth rates and gain to feed ratios followed the same pattern.

The authors conclude that the evidence from the above three experiments is overwhelmingly in favor of the concept that differences in growth rate of chicks do not, per se, necessitate different dietary levels of lysine for optimum growth. According to the authors, the findings further support H. J. Almquist's view (*J. Nutrition* **34**, 543 (1947)) that the proportions of indispensable amino acids remain the same for any suboptimal rate of growth. Lower feed consumption would satisfy the lower absolute requirement for lysine of a slow-growing chick without changing the requirement of lysine as a percentage of the feed.

According to the authors, the lower growth rate and lower requirement found by Edwards *et al.* (*loc. cit.*) with the wheat gluten diet is probably caused by the amino acid deficiency in the test diet, without a

cause and effect relationship between growth rate and lysine requirement. Thus since the low growth rate appears to result from the lack of ample amounts of balanced protein, the statement regarding the effect of a low growth rate on amino acid requirement does not appear justified.

Further strengthening the contention that rate of growth does not necessarily influence the percentage requirement for essential amino acids are the studies of H. H. Mitchell and J. R. Beadles (*J. Nutrition* **47**, 133 (1952)), which show that the protein percentage for maximum growth of rats was independent of caloric intake level when the caloric density of the diet remained the same. D. E. Becker *et al.* (*J. Animal Sci.* **13**, 346 (1954)) found that a moderately depressed feed intake of pigs did not influence the protein requirement, and H. D. Hutchinson (*Doctoral Thesis, Univ. of Illinois* (1957)) observed that, when lysine was the limiting factor in the basal ration, neither the level of feed intake nor the consequent difference in growth rate influenced the lysine requirement of the weanling rat.

Other authors have offered evidence suggesting that different breeds may have differing amino acid requirements. D. M. Hegsted *et al.* (*J. Biol. Chem.* **140**, 191 (1941)) concluded that higher arginine and glycine levels were required for fast-feathering breeds of chicks during times of feather development. M. W. McDonald (*Australian J. Agr. Res.* **9**, 161 (1958)) attributed his findings on Australorps to a specific metabolic block in sulfur amino acid metabolism rather than to a difference in growth rate between breeds.

## COLD AND RIBOFLAVIN REQUIREMENT

*The requirements for riboflavin are proportional to intake of food even under exposure to cold.*

The concept that the requirements for riboflavin and other B vitamins are propor-

tional to intake of food has been challenged by H. H. Mitchell *et al.* (*J. Nutrition* **41**,

317 (1950)) and by B. H. Ershoff (*Proc. Soc. Exp. Biol. Med.* **79**, 559 (1952)) who subjected their experimental animals to low temperatures. Riboflavin is known to be involved in the production of the adrenocortical hormones, which are in turn concerned with the physiological response to cold. Moreover, riboflavin plays a coenzymatic role in the catabolism of fat, the importance of which may be increased by the presence of cold.

However, despite this evidence and reasoning, O. A. Bessey *et al.* (*J. Nutrition* **64**, 185 (1958)) found that there was no increase in riboflavin utilization by riboflavin-deficient rats when metabolism was increased by exposure to cold. M. Fontaine and A. Raffy (*C. R. Soc. Biol.* **136**, 297 (1942)) are quoted by these workers as observing that rats exposed to cold had a 20 per cent higher concentration of riboflavin in their livers.

D. A. Vaughan and L. N. Vaughan (*J. Nutrition* **68**, 485 (1959)) applied a more critical test to the idea that the requirement of rats for riboflavin might be dependent on factors other than food intake. They reasoned that if food intake is the most critical determinant of riboflavin needs, animals subsisting in the cold, when allowed to fill their cold-induced energy requirements, should be as well off as their controls at normal temperature when eating the same levels of riboflavin per unit of food.

To test this hypothesis, they used two groups of 25 male Sprague-Dawley rats ranging in weight from 175 to 250 g. One group was placed in individual cages in a cold room held at  $5 \pm 2^\circ \text{C}$ . The other group was maintained at  $25^\circ \text{C}$ . All rats were placed on a riboflavin-deficient diet until growth ceased or fluctuated about a central point. The deficient diet consisted of: vitamin test casein (18 per cent), sucrose (68 per cent), vegetable oil (10 per cent), and U.S.P. Salt Mixture No. 2 (4 per cent). The vitamin mixture supplied: 2000 units of vitamin A, 222 units of vitamin D, 11.1 mg.

of alpha-tocopherol, 100 mg. of ascorbic acid, 11.1 mg. of inositol, 166.5 mg. of choline chloride, 5 mg. of menadione, 11.1 mg. of para-aminobenzoic acid, 10 mg. of niacin, 22.2 mg. of pyridoxine hydrochloride, 6.66 mg. of calcium pantothenate, 44 micrograms of biotin, 200 micrograms of folic acid, 3 micrograms of vitamin B<sub>12</sub>, and 2.22 mg. of thiamine hydrochloride per 100 g. of diet.

At the end of 28 days, growth had ceased in both groups of animals, at which time each group of 25 rats was divided into five sub-groups receiving 0.5, 1.0, 1.5, 2.0 or 4.0 micrograms of riboflavin per gram of diet (4.0 micrograms per gram was assumed adequate for maximum growth).

It was found that the growth response of rats receiving the same level of riboflavin in the diet was unaffected by the environmental temperature when the riboflavin level was 2.0 micrograms or less. At the 4.0 micrograms per gram level, however, the growth of the rats kept at normal temperature was significantly greater than that of corresponding rats maintained in the cold.

These results do not agree completely with those of Mitchell *et al.* (*loc. cit.*) who observed that in growing pigs the riboflavin requirement per unit of food was greater at  $5^\circ$  than at  $29^\circ \text{C}$ . Ershoff (*loc. cit.*) also reported impaired survival and depressed growth in rats subjected to cold. Vaughan and Vaughan explain, however, that in Ershoff's experiments the animals were already deficient when placed in the cold room, while their animals were allowed to become deficient at  $5^\circ \text{C}$ , a procedure permitting them to become adjusted to cold before being subjected to the additional stress of the deficiency.

Both the level of riboflavin in the food and exposure to cold had a significant effect on food intake, but there was no significant interaction between these conditions. Food intake rose rapidly with increasing levels of riboflavin until the 1.5 microgram level was reached, whereupon the increment became

quite small. The differences in food intake between normal and cold rats (cold rats ate more) were quite uniform across successive levels of riboflavin. Apparently the portion of the appetite stimulated by the exposure to cold was not affected by the anorexia induced by the deficiency in riboflavin.

When the final weights of the rats were adjusted for food intake and initial weight,

it was obvious that the rats held at 25° C used their food more efficiently for growth. In addition, multivariate analysis showed that the level of riboflavin had a significant effect on the adjusted final weights, indicating that the efficiency of food utilization for growth, as well as food intake, was lowered in riboflavin-deficient rats at both temperatures.

## PHYSIOLOGICAL PROPERTIES OF DITHIOPROPYLTHIAMINE

*Dithiopropylthiamine produces higher blood and urine thiamine and higher liver cocarboxylase than does thiamine itself by virtue of its greater absorption rate.*

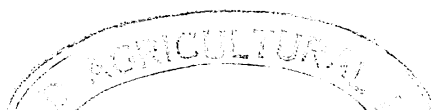
Allithiamine is the derivative formed by the reaction of allicin (diallyl disulfide, monosulfoxide) in the gut or the test tube, with the ring sulfur of thiamine. The ring is thus opened and the allyl thiol radical becomes attached to thiamine in a disulfide linkage. Allicin itself is formed from alliin (s-allylhomoecysteine sulfoxide) by the enzyme alliinase, both enzyme and substrate occurring in the garlic (*allium sativum*) and certain related species. M. Fujiwara and his co-workers found, in a series of experiments, that allithiamine is more rapidly absorbed from the intestine than is thiamine itself and thus gives higher blood levels of thiamine with greater rapidity (apparently, allithiamine undergoes ring closure to thiamine in the body) (*Nutrition Reviews* 16, 336 (1958)).

In a further attempt to study the physiological properties of compounds of this type, D. Hioco, R. Tixier and A. Uzan (*Bull. Soc. Chim. Biol.* 41, 1075 (1959)) undertook experiments on animals and man with dithiopropylthiamine, a compound with the same structure as allithiamine but with the propyl replacing the allyl group.

Three types of animal experiments were carried out. In the first, doses of 100 to 10,000 micrograms of either dithiopropylthiamine or thiamine hydrochloride were given orally to rats, and their liver cocarboxylase (thiamine pyrophosphate) was

measured after one hour. Control animals, which had received no supplement, had an average of 6 micrograms of cocarboxylase per gram of liver. The highest value obtained using thiamine itself was about 10, while that for dithiopropylthiamine was 20 micrograms per gram. In the second experiment, 250 micrograms of both compounds were given orally to the rats and the cocarboxylase was measured at one, two, four and six hours. The liver cocarboxylase rose more rapidly with the dithiopropylthiamine and to about twice the level obtained with thiamine itself. In the third experiment, the duration of the effect was studied by measuring liver cocarboxylase six hours after ingestion of either thiamine or dithiopropylthiamine. As before, the levels following dithiopropylthiamine were about twice those following thiamine regardless of dose size.

In the human experiments, a 100 mg. of dithiopropylthiamine or thiamine hydrochloride were given orally to four or five subjects and the blood levels of thiamine were measured at one and one-half and four hours. Little rise above normal was found after thiamine ingestion whereas, after ingestion of dithiopropylthiamine, elevation to between 15 and 100 micrograms per 100 ml. of blood occurred at 90 minutes and levels between 10 and 30 micrograms were observed even four hours after dosage. Moreover, urine levels, which are a reflection of



intestinal absorption rate, were several times higher with dithiopropylthiamine than with thiamine.

It has thus been shown that this derivative of thiamine, by virtue of its rapid in-

testinal absorption, causes far more rapid attainment of high blood levels of thiamine and of liver cocarboxylase than does thiamine itself. Its low toxicity, when compared with thiamine itself, also recommends it.

### VITAMIN SPARING BY POORLY DIGESTED CARBOHYDRATES

*Poorly digested sugars promote proliferation of intestinal bacteria which synthesize sufficient B-vitamins for the needs of mammals.*

It has been shown by T. B. Morgan and J. Yudkin (*Nature* **180**, 543 (1957); *Nutrition Reviews* **16**, 126 (1958)) that rats on a diet devoid of the B-vitamins can thrive if the diet contains 20 per cent sorbitol. In order to study this phenomenon further, H. Haenel, H. Ruttloff and H. Ackermann (*Biochem. Z.* **331**, 209 (1959)) have carried out experiments designed to confirm and amplify the original observations.

Four groups of weanling rats were placed on the following diets: (1) a basic diet consisting of 55 per cent sucrose, 15 per cent cocoa butter, 20 per cent casein, 5 per cent cellulose, 5 per cent salt mixture and adequate proportions of the vitamins mixed daily with the diet; (2) the basic diet without the B-vitamins; (3) the basic diet without the B-vitamins but with 20 per cent sorbitol replacing a like amount of sucrose; (4) a natural diet (grains, milk powder, etc.).

The animals on the natural diet gained the most weight (to 180 g. at 60 days), followed by the vitamin-supplemented group (to 150 g.). The vitamin-deficient group lost weight and all had died within 45 days. Inclusion of 20 per cent sorbitol in this diet, however, prevented obvious vitamin deficiency diseases and permitted some weight gain (to 135 g.). These results confirmed the previous report of Morgan and Yudkin (*loc. cit.*) in most respects. However, the sorbitol-fed animals did not grow quite normally and responded irregularly to the treatment in that a few gained no weight and died with symptoms of vitamin deficiency. Moreover, addition of sorbitol to

the diet of the deficient animals brought about slow recoveries as compared with a B-vitamin supplement.

Microbiological analyses of the urines confirmed the beneficial effect of the sorbitol. Urine contents (reflecting blood levels) of thiamine, niacin, riboflavin and vitamin B<sub>6</sub> were lowest for the vitamin-deficient group, intermediate for the sorbitol group and highest for the vitamin-supplemented group. The implication was thus that sorbitol, in some way, was effecting higher concentrations of these vitamins in the intestines and, hence, in the blood of the deficient animals.

Analysis of 24-hour urine samples of all groups of rats revealed that of those on the artificial diets, the sorbitol-supplemented group had the highest concentrations of indican (indoxyl sulfate, a product of bacterial alteration of indole, indicative of greater bacterial action). The group on the natural diet, however, had somewhat higher indican concentrations.

Further experiments showed that lactose, also, had some vitamin-sparing action, although it was only about half that of sorbitol. Investigation of the rate of absorption of these two carbohydrates revealed that they persisted throughout the small intestine, whereas the readily absorbable sugars (glucose, sucrose and fructose) disappeared in the top portion of the duodenum.

These results led the authors to what appeared to be the obvious conclusion; that those carbohydrates that are absorbed with difficulty promote the proliferation of intestinal bacteria which synthesize the B-



vitamins for absorption by the host animal.

This conclusion is also shared by L. A. Cherkes and A. A. Dinerman (*Biokhimiya* 24, 329 (1959)), who studied the effect of dietary carbohydrate on development of choline deficiency in rats. On the basic choline-deficient diet (consisting of peanut-cake meal, 30 per cent; casein, 6 per cent; lard, 18 per cent; sucrose, 40 per cent; gelatin, 3 per cent; salt mixture, 4 per cent and adequate amounts of B-vitamins) weanling rats developed severe hemorrhagic degeneration of the kidneys, one of the typical symptoms of this state (W. H. Griffith, *Biological Action of the Vitamins*, p. 169. Univ. of Chicago Press (1942)).

Substitution of 20 per cent sorbitol and 20 per cent lard for the sucrose in a second group of rats resulted in almost complete elimination of the kidney damage. The diarrhea accompanying this diet, however, was so severe that a third group of rats was gradually adapted to the sorbitol diet by stepwise replacement of the sucrose by sorbitol. In this group, three of 11 animals showed moderate to severe kidney damage.

Examination of the three groups of rats for fatty infiltration of the livers revealed a

somewhat different picture. The first group, as was expected, showed severe fatty infiltration. The second group had considerably lower liver fat, but it was thought that this might be from the effect of the diarrhea, which lowered the caloric intake and reduced the weight gain. This is highly probable since the third group, which did not have diarrhea and showed normal weight gains, had liver fat contents about as high as those of the first group.

The authors concluded that the amount of choline furnished by the influence of the sorbitol, while sufficient to prevent kidney damage, fell short of the requirement needed to prevent fatty livers. They should also have considered that their sorbitol-containing diets were very high in lard as compared to the sucrose diet and that this factor alone might have influenced the development of fatty livers.

Whether this knowledge can be put to practical use in human nutrition has not been determined, but it undoubtedly represents an outstanding example of the importance of intestinal bacteria in the nutrition of mammals, and should be considered in all such experiments.

### LIME-TREATED CORN

*Alkali or lime treatment converts the bound niacin in corn, unavailable to animals, to a free available form, probably explaining the absence of pellagra in countries where tortillas are eaten in quantity.*

Although corn is the primary staple in those parts of the world where pellagra is endemic, it has been recognized for a number of years that corn contains considerable amounts of niacin. It was observed that only a part of the niacin could be extracted from corn with water, which led to the suggestion that niacin existed in a free and a bound form. The latter was believed unavailable to man and animals (*Nutrition Reviews* 15, 53 (1957)).

The absence of pellagra in such corn-eating countries as those in Central America

was attributed to the liberation of the bound niacin during the preparation of the corn for domestic use. The lime treatment of the corn, used universally in that part of the world, presumably converted the bound niacin to the free form, thus increasing the amount of niacin available to the consumer.

A number of studies have been made to assess the efficacy of the lime treatment in converting corn to an anti-pellagragenic food. One of these was the study by G. A. Goldsmith and co-workers (*Nutrition Reviews* (loc. cit.)). These investigators, however,

failed to find any difference respecting pellagra-curing activity between corn made into tortillas, as in Central America, and corn prepared according to the methods used in the southern part of the United States when that area had a high incidence of pellagra. The investigators pointed out that the primary limitation of their study was the small amount of corn in the diet fed the subjects (15 to 20 per cent of the calories). In Central America tortillas are a major constituent of the diet and it is possible that the large amount of lime-treated corn used in that part of the world may increase the intake of free niacin to such an extent that it prevents pellagra.

For many years, E. Kodicek has studied the availability of niacin in different foods. As early as 1940 (*Biochem. J.* **34**, 712 (1940)), when he was studying the conditions that influence the cyanogen bromide procedure, he found that in such animal tissues as liver and muscle all of the niacin could be extracted with boiling water. However, only a small fraction of the material in corn which gave the color test for niacin could be extracted with water. On the other hand, hydrolysis with dilute alkali extracted so much niacin from corn that, if it were all true or active niacin, "yellow maize which is known to be deficient would have a high concentration of nicotinic acid."

Physiological verification that only a small part of the niacin in corn is active or free came from Kodicek's comparison of the rate of production of blacktongue in dogs receiving either a corn or a purified diet. Under these circumstances the corn diets, which contained from 12 to 27 micrograms of niacin per gram by chemical analysis after alkaline hydrolysis, had the same effect as a purified diet containing only 7 micrograms of niacin per gram.

On the basis of work such as this, many studies were carried out with animals fed corn which had been soaked in dilute sodium hydroxide or lime water. These animals did not develop symptoms of a niacin defi-

ciency, whereas the controls fed the untreated corn did (Kodicek, R. Braude, S. K. Kon and K. G. Mitchell, *Brit. J. Nutrition* **13**, 363 (1959)).

Since most of the preceding work had been done with rats and chicks, Kodicek, Braude, Kon and Mitchell (*Brit. J. Nutrition* **10**, 51 (1956)) tested the alkali-hydrolyzed corn as a curative agent for niacin-deficient pigs. In so far as niacin requirements are concerned, the pig more closely resembles man than does the rat. Analysis of the milling fractions indicated that 73 per cent of the total niacin in corn occurred in the hominy meal and bran, which represented 27 per cent of the corn. In view of this fact, 265 pounds of the mixture of hominy meal and bran were treated with 400 liters of 0.5 N-sodium hydroxide at a temperature of 60°. The alkali was neutralized with 10 liters of concentrated hydrochloric acid and then the mixture was dried in a heating tunnel at 70°. The dried, hydrolyzed mixture was mixed with the remainder of the corn and incorporated at a level of 78 per cent in a diet containing 10 per cent pea meal, 5 per cent casein, 3 per cent cod liver oil, and 2.5 per cent salts.

When pigs weighing 37 to 59 pounds were maintained on a diet similar to the above except that untreated corn was used in place of the alkali-treated corn, the animals stopped growing and showed symptoms of a niacin deficiency in about 35 days. At this time, six pigs were supplemented with 6 mg. of niacin per day. Another six were changed to the diet containing the alkali-treated corn. Both groups of pigs immediately began to gain weight, with the niacin-supplemented ones gaining  $5.0 \pm 0.33$  pounds per week and those fed the alkali-treated corn gaining  $3.2 \pm 0.62$  pounds.

Chemical analysis and paper chromatography confirmed the fact that all of the niacin in the untreated corn was in the bound form, whereas all in the alkali-treated corn was in the free form (Kodicek *et al.*, *loc. cit.*).

To round out the above studies, Kodicek

and co-workers (*Brit. J. Nutrition* **13**, 363 (1959)) repeated their work with corn treated with lime water according to the method used in Mexico for preparing tortillas. One ton of corn was so treated and then baked in the form of flat cakes to make tortillas. Another three tons of corn from the same batch were used in the diets fed to the pigs to produce the deficiency state.

Chemical analysis indicated that the untreated corn contained 17.7 micrograms per gram of niacin, but that only 0.3 micrograms of this was in the free form. The tortillas, on the other hand, contained 11.0 micrograms of niacin per gram of dry material, all of which was in the free form. The lime treatment resulted in a reduction of the thiamine content of the corn from an original value of 4.6 micrograms per gram to 0.39. The riboflavin was reduced from 0.43 micrograms per gram to 0.27, calcium pantothenate from 3.4 to 1.1 micrograms, vitamin B<sub>6</sub> from 3.4 to 1.7 micrograms, folic acid from 70 to 8 millimicrograms, and the tocopherols from 16.0 to 4.6 micrograms. Synthetic vitamins were added to the diet containing tortillas to compensate for these losses.

Eighteen weanling pigs were maintained on the untreated corn diet until they ceased to gain or lost weight for seven days. Six pigs were then maintained on the diet as controls. Two of these animals died showing typical signs of a niacin deficiency, while the other four gained weight at the rate of  $1.7 \pm 1.43$  pounds per week. Another six pigs received 6 mg. of niacin per day, which

was in addition to the 27.5 mg. each one received from the diet. Of the latter, only 3.4 mg. was in the free form, making a total intake of 4.4 mg. of free niacin per day. These animals gained  $6.5 \pm 0.42$  pounds per week. The six pigs on the tortilla diet received 19.9 mg. of niacin per day, all of which was in the free form, and these animals gained  $7.9 \pm 1.15$  pounds per week.

There was a correlation coefficient of  $+ 0.86$  between the intake of free niacin and the gain in body weight; the coefficient for total niacin was  $+ 0.60$ . Although the two correlation coefficients did not differ significantly, the higher correlation coefficient for the free niacin suggested to the investigators that the substance in the tortillas responsible for the body weight gains was the free niacin.

At the end of the experiment, the animals were killed and the livers assayed for niacin. Here again there was a correlation coefficient of  $+ 0.81$  between the intake of free niacin and the content of this vitamin in the liver. In this case, the correlation coefficient for the total niacin was  $+ 0.30$ . These two correlation coefficients differed significantly.

The investigators concluded that the beneficial effect of tortillas in curing niacin deficiency in pigs could be attributed entirely to the conversion of bound niacin in the corn to the free form as a result of the lime treatment. They present considerable evidence to substantiate their conclusion that changes in availability of proteins or of amino acids could not explain the difference between the corn before and after lime treatment.

## ENZYME CHANGES IN GALACTOSE CATARACT

*Both in vivo and possibly in vitro galactose-1-phosphate inhibits the activity of glucose-6-phosphate dehydrogenase activity in rat lenses.*

One of the experimental methods of producing cataracts in the eyes of animals, a condition where the lens becomes opaque or clouded, is to maintain them on diets with a high content of galactose. Moreover,

infants with a hereditary disorder termed galactosemia may develop cataracts of the eye (*Nutrition Reviews* **14**, 5 (1956)). H. M. Kalckar and associates (*Science* **125**, 105, 113 (1957)) noted that the liver and red

blood cells of patients with galactosemia have a relative and absolute decrease in the activity of the enzyme galactose-1-phosphate uridyl transferase. This enzyme converts galactose-1-phosphate to a glucose-1-phosphate. It has also been noted by V. Schwarz and L. Golberg (*Biochim. et Biophys. Acta.* **18**, 310 (1955)) that there is an accumulation of galactose-1-phosphate in the lens capsule and epithelium of rats maintained on a diet containing 30 per cent galactose. There was about ten times the concentration of galactose-1-phosphate in the lenses of the rats fed galactose as compared to the lenses of normal rats. In the metabolism of carbohydrate by the lens, J. H. Kinoshita (A.M.A. Arch. Ophth. **54**, 360 (1955)) reported that the hexose monophosphate shunt is of considerable importance.

Recently, S. Lerman (*Science* **130**, 1473 (1959)) studied the possibility that galactose-1-phosphate might directly inhibit some steps in the metabolism of glucose. To do this he assayed the levels of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the lenses of normal rats and those fed high levels of galactose. Twelve animals were in each of the two groups. When the first signs of cataract formation were detected by ophthalmoscopic examination (peripheral subcapsular vacuolization) in the animals fed the galactose, both groups were sacrificed and the content of these dehydrogenases was determined in the lenses. This defect usually occurred on the twelfth to fourteenth day after beginning the galactose feeding.

The level of the glucose-6-phosphate dehydrogenase in the lenses of the control animals averaged 25.2 units (23.7 to 26.9), while the level of this enzyme in the lenses of the animals fed the galactose averaged 13.8 (7.9 to 18.8) units. Thus there was a decrease of approximately 45 per cent in the activity of glucose 6-phosphate dehydrogenase in the lenses of the animals consuming galactose. No differences were noted in the enzyme activity of 6-phosphogluconate

dehydrogenase between the animals fed galactose (6.25 units) and those receiving glucose (6.16 units).

In another series of studies, Lerman maintained two groups of 14 animals each on the diets described above and sacrificed one pair of control (glucose-fed) and one pair of test animals (galactose-fed) on the fourth, sixth, seventh and eighth day of the feeding schedule. The remaining pairs of animals were sacrificed on the sixteenth, nineteenth and twentieth day of the study. Cataracts were present by the end of the study. The lenses of each animal were assayed for both glucose-6-phosphate and 6-phosphogluconate dehydrogenases.

There was a progressive fall in the level of glucose 6-phosphate dehydrogenase activity in the lenses of the animals receiving the galactose diets. The difference was noted by the fourth day of feeding and by the fourteenth day the activity fell to about 60 per cent of normal, remaining at this decreased level until the end of the study. No difference was noted between the levels of 6-phosphogluconate dehydrogenase in the two groups of animals. Thus it appeared that there was an inhibition of the glucose-6-phosphate dehydrogenase activity *in vivo* when the animals were fed the galactose.

Lerman then undertook studies to determine whether an inhibition could be produced *in vitro*. Lenses from five normal rats were studied. One pair of lenses was homogenized in 2 ml. of distilled water and the insoluble protein removed by centrifugation. Half of each portion of the soluble protein fraction was incubated in a media containing an excessive amount of galactose-1-phosphate compared with glucose-6-phosphate. The remaining portion of the lens homogenate was incubated with glucose-1-phosphate as substrate. After three hours of incubation at 37° C an aliquot was assayed for the combined glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activity. By comparing the two

groups, it was found that there was an average inhibition of 21 per cent (16 to 35 per cent) when an excess of galactose-1-phosphate was present in the media. It should be noted, however, that only five animals were used in this preliminary study and the range of inhibition was broad.

Since all these aliquots contained some glucose-6-phosphate, another study was set up with the soluble homogenate derived from four sets of normal rat lenses, one half of which was incubated with 15 micromoles of galactose-1-phosphate and the other half with a similar amount of sucrose. There

was an average inhibition of 20 per cent of the activity of the glucose-6-phosphate dehydrogenase while the 6-phosphogluconate dehydrogenase was not inhibited.

This investigator noted that galactose-1-phosphate is capable of inhibiting specifically glucose-6-phosphate dehydrogenase *in vivo* and may also do it *in vitro*. Thus the inhibition of the hexose monophosphate shunt is implicated in the pathogenesis of experimental galactose cataracts in rats. A similar mechanism may also be involved in the cataracts occurring in infants with galactosemia.

### GASTRIC HYPERTROPHY IN FASTED RATS

*Adaptation to intermittent starvation permitted 132 g. rats to consume 56 g. of feed during one night following three days of fasting. After 17 weeks of such a regimen the stomach was markedly increased in size with hypertrophy of the walls.*

When individuals who are accustomed to eating a diet containing a fairly large percentage of fat are given one that is low in fat, a number of days ensue before they are able to adequately increase their food intake. During this period of adaptation there occurs an increase in the capacity of the stomach, after which time the individual is able to consume a volume of food which ordinarily would have caused him a great deal of distress.

A somewhat analogous adaptation has been studied in rats by E. Holeckova and P. Fabry (*Brit. J. Nutrition* **13**, 260 (1959)). These investigators subjected 100 to 200 g. female rats to increasing periods of starvation. The fasts, however, were interspersed by single days when the animals had free access to a mixed diet containing 53 per cent of the calories from carbohydrate, 25 per cent from protein and 22 per cent from fat. When 24 rats were starved every alternate day, their body weight increased slightly during the first week but then plateaued for the remaining three weeks. When these same rats were fed only three

days each week, their body weight again increased slightly, but plateaued during the third week, remaining constant for the next four weeks. However, when these rats were fed only twice a week, there was a gradual loss of body weight which leveled out at the original weight before starvation.

The control rats that were fed the diet on an ad libitum basis showed a variation in daily food intake ranging from 15 to 20 g. per rat per day. When the weekly food consumption was computed on a per diem basis for the intermittently starved rats, there was a progressive increase throughout the experimental period. The values for the latter group increased from approximately 10 g. per rat per day at the start of this period to 15 g. at the end.

As a result of intermittent feeding, the rats showed a tremendous increase in their capacity to consume food whenever it was available. The amount of feed each rat consumed on the day it was fed increased from 16 g. at the start of the study to 50 g. at the end. This adaptation permitted the intermittently starved rats to consume 112

g. of food in only two days during the last week of the study, while the non-starved rats ate 133 g. in the seven days.

The intermittently starved rats not only consumed more food during a given time than the controls, but retained food in their stomachs for a longer period of time. During the night following three days of starvation, the rats consumed 56 g. of food. X-rays of these 130 g. rats revealed that the stomach occupied the greater part of the abdomen. Twenty-four hours later, during which no food had been available to them, their stomachs still contained much more food than the controls that had been eating 20 g. of food per day.

The average weight of the stomachs in the 18 rats that had been intermittently starved for six weeks was  $988 \pm 42$  (S.E.) mg. The body weight of these rats averaged  $132 \pm 4.9$  g. The average weight of the stomachs in the 18 control rats was  $937 \pm 30$  mg.; the average body weight was  $177 \pm 4.4$  g. After 17 weeks of intermittent starvation, the average stomach weight for the 24 rats was  $1450 \pm 62$  mg. for the rats weighing on an average  $173 \pm 5.2$  g. In the controls, the average stomach weight was  $1146 \pm 33$  mg., while the body weight was  $234 \pm 6.6$  g. The major increase in the size of the stomach

occurred in the "translucent" part (presumably the cardiac portion). The increase in the "opaque" part (presumably the pyloric portion) of the stomach was relatively small. Histological examination showed hypertrophy of all parts of the stomach, but it was most pronounced in the translucent part.

Holeckova and Fabry mentioned in their discussion additional publications not available to the reviewer. Therein they reported that the weight of the small intestine was also increased in intermittently starved rats. Associated therewith was an increased absorption of glucose and an increased alkaline phosphatase activity. The hypertrophy of the stomach and small intestine and the increased enzyme activity occurred in spite of the fact that other organs of the animal and the body as a whole lost weight.

This is an interesting and unique study on the effect of adaptation in the animal to periods of starvation. It suggests that additional studies and observations should be made of similar conditions in man, especially in those individuals subjected to marked changes in dietary practices. These changes are likely to appear in individuals who heed the advice of food faddists recommending such diets as those very low in fat.

### ASCORBIC ACID IN THE PARENTAL DIET AND CALCIUM UTILIZATION BY THE CHICK

*Factors other than vitamin D may sustain normal intestinal absorption of calcium in the chick.*

The presence of ascorbic acid in the maternal diet influences the gastrointestinal absorption of calcium by chicks, whereas vitamin D is not involved primarily with intestinal absorption of calcium but rather is essential for normal bone development. These conclusions by P. A. Thornton, C. W. Weber and R. E. Moreng (*J. Nutrition* **69**, 33 (1959)) are based on studies arising out of unusual observations made during the investigation of rates of feed movement in

the intestinal tract and of blood concentrations and tibial uptake of  $\text{Ca}^{45}$  in control and rachitic chicks (the progeny of parents fed a ration containing ascorbic acid, a nutrient synthesized by the chicken and hence regarded as a nonessential dietary constituent).

Previous investigations (R. Nicolaysen and N. Eeg-Larsen, *Vitamins and Hormones* **11**, 29 (1953); G. H. Bourne, *The Biochemistry and Physiology of Bone*, p. 561. *The*



Academic Press, Inc., New York (1956); J. M. Leichsenring, L. M. Norris and M. L. Halbert, *J. Nutrition* **63**, 417 (1957)) have considered ascorbic acid and vitamin D separately with respect to calcium absorption and metabolism. The present authors, on the other hand, have begun to investigate the influences of ascorbic acid and vitamin D on calcium absorption and metabolism by studying the two vitamins simultaneously.

The works reviewed by Nicolaysen and Eeg-Larsen (*loc. cit.*) have shown experimentally that vitamin D is necessary for the intestinal absorption of calcium or phosphorus or both and, in addition, for normal bone deposition. In other words, it has been suggested that vitamin D functions in promoting normal bone development either by increasing calcium and phosphorus absorption or by promoting calcium and phosphorus metabolism after absorption. The studies reviewed by Bourne (*loc. cit.*) have reported both an increase and no increase in retention of calcium by rats fed orange juice and ascorbic acid and have suggested that calcium may be absorbed from the intestine as calcium ascorbate. Leichsenring, Norris and Halbert (*loc. cit.*) have shown that both orange juice and crystalline ascorbic acid increase calcium retention in women.

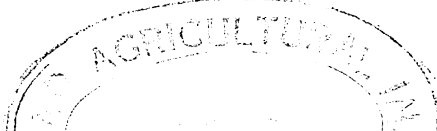
In the present experiments, Thornton, Weber and Moreng used New Hampshire-Delaware cross chicks which were fed ground milo until three days of age when they were divided in a random manner, group one being given a vitamin D<sub>3</sub>-deficient ration, and group two the same ration supplemented by 13,500 I.C.U. of vitamin D<sub>3</sub> per 100 pounds of ration. The deficient ration contained the respective percentages of the following ingredients: ground milo, 70; soy bean oil meal, 25; ground alfalfa meal, 1.50; ground limestone, 0.50; steamed bone meal, 2.50; iodized salt, 0.50; manganese sulfate supplement, 0.022; and vitamin mix, 0.113. In about three weeks, the deficient

ration produced the rachitic symptoms of an ungainly walk, softness of the beak, unthrifty appearance and slow growth. Ten chicks were used in each control and experimental group with an equal distribution when possible between males and females.

At four weeks of age, the chicks received directly into the lumen of the crop injections of 0.5 ml. of a solution containing approximately 10 microcuries of Ca<sup>45</sup> (in the form of CaCl<sub>2</sub> in hydrochloric acid solution) plus 15 mg. of Evans Blue dye. The birds were sacrificed by ether-induced asphyxiation at 15, 30, 45 and 60 minutes following injection. Just prior to sacrifice, a sample of heart blood was collected in a tube containing an 0.2 per cent solution of sodium oxalate. After sacrifice the non-calcium-absorbing "upper bowel" (esophagus, crop, proventriculus and ventriculus) were removed as a unit and prepared for radioassay. The tibiae were removed and measurements made of the epiphyseal-cartilaginous plates. The "upper bowels" and tibiae were ashed at 700° C, the ash taken up in 25 per cent HNO<sub>3</sub> solution, brought to a constant volume and an aliquot assayed for Ca<sup>45</sup> activity in a gas flow, windowless type scaler. Statistical differences between groups were determined by calculating the standard error and applying the "t" test to such data.

The data acquired in these studies were different from those reported previously by B. B. Migicovsky and J. W. S. Jamieson (*Canad. J. Biochem. Physiol.* **33**, 202 (1955)) and by K. W. Keane, R. A. Collins and M. B. Gillis (*Poultry Sci.* **35**, 1216 (1956)). These authors previously reported that the specific activity of Ca<sup>45</sup> in the blood reached a maximum in control chicks ten minutes after injection, declining progressively thereafter, while specific activity in the blood of deficient chicks reached a maximal activity in 30 to 90 minutes but was at a much lower level than in the controls.

Thornton, Weber and Moreng, on the other hand, observed that Ca<sup>45</sup> blood levels, expressed as per cent of Ca<sup>45</sup> recovered, had



risen sharply in both control and deficient chicks by 15 minutes after injection. They explain this by the rapid, exponential rate of feed movement from the "upper bowel" to the absorptive "lower bowel". At 30 and 45 minutes,  $\text{Ca}^{45}$  blood levels were similar to those at 15 minutes, but at 60 minutes  $\text{Ca}^{45}$  blood levels in both groups were again increased. The amount of  $\text{Ca}^{45}$  deposited in the tibiae were markedly different between control and deficient groups at 60 minutes. These differences were directly correlated with the amount of  $\text{Ca}^{45}$  leaving the upper bowel, the deficient group failing to send enough calcium from the "upper" to the absorptive "lower" bowel.

In seeking explanations for the differences, the authors observed that their experiments resembled those of Migicovsky and Jamieson (*loc. cit.*) and Keane, Collins and Gillis (*loc. cit.*) except that ascorbic acid had been added to the parental diet in the authors' experiments. The correlation between the amount of  $\text{Ca}^{45}$  leaving the "upper bowel" and that recovered from the tibiae in chicks from parents not receiving ascorbic acid was not significant for the deficient chicks but was highly significant for the control groups, whereas the correlation in chicks from parents receiving ascorbic acid was highly significant in both control and deficient chicks.

These observations led Thornton, Weber and Moreng to set up a "pilot study" to observe chicks from hens receiving 0, 10, 20, or 40 mg. of ascorbic acid per pound of ration. The tibial uptake of  $\text{Ca}^{45}$  was increased (to a highly significant degree, statistically) in vitamin  $\text{D}_3$ -deficient females

when the parents received ascorbic acid. Epiphyseal plate widths were wider in both females and males. There was a good correlation between epiphyseal plate widths, tibial ash weights and the amount of  $\text{Ca}^{45}$  taken up by the tibiae in the chicks from unsupplemented parents but none in chicks from supplemented parents.

The presence of ascorbic acid in the parents' diet seemed, in the authors' opinion, to have increased the severity of rickets despite the chicks' ability to deposit more calcium in bone.

In view of their data, original and derived, the authors offer four suggestions: (1) calcium absorption per se will not necessarily prevent rachitic symptoms; (2) the primary function of vitamin  $\text{D}_3$  is not necessarily the promotion of intestinal absorption of calcium; (3) ascorbic acid or some agent produced by the presence of this vitamin in the parental diet helps maintain calcium in rachitic progeny; and (4) the presence of of this vitamin in the parents' diet may increase the degree of rickets in progeny given vitamin  $\text{D}_3$ -deficient diets.

The authors' investigations of the role of ascorbic acid in the parental diet seem to have arisen incidentally and hence for the moment have a flavor of retrospective research. Nonetheless, they are provocative and challenge some fundamental concepts of calcium metabolism. Further investigations extending their pilot study of chicks and, hopefully, of other species including the guinea pig will be anticipated. Opportunities for comparable studies in man when suitable clinical situations are presented should be anticipated.

## NOTES

## Letters to the Editor

Dear Sir:

We have read the article "Vitamin K Deficiency in Newborn Infants" (*Nutrition Reviews* 17, 229 (1959)) and should like to call your attention to the statement therein in which our paper "The Relation of Vitamin K Deficiency to Hemorrhagic Disease in the Newborn" (H. Dam, H. Dyggve, H. Larsen and P. Plum, *Advances in Pediatrics* 5, 129 (1952)) is mentioned as supporting the idea that prophylactic vitamin K administration to mothers or infants is of doubtful value in the newborn period.

This quotation is not in accordance with the content of our paper, and we do not understand how our paper could have led authorities to state that the value of routine prophylactic vitamin K administration was not clearly established (*Recommended Dietary Allowances, National Academy of Sciences-National Research Council Publication 589 (1958)*), as mentioned in the article in *Nutrition Reviews*.

Our studies, described in *Advances in Pediatrics* 5, 129 (1952)), demonstrate the efficacy of antenatal vitamin K prophylaxis against decrease of prothrombin activity and several forms of hemorrhage in the infant in the period immediately after birth.

HENRIK DAM  
HOLGER V. DYGGVE  
E. HJALMAR LARSEN  
PREBEN PLUM  
Copenhagen, Denmark

*Editor's Note:* It was not the work of H. Dam *et al.* but the work of Edith L. Potter (*Am. J. Obstet. Gynec.* 50, 235 (1945)) which led some authorities to state that the value of routine prophylactic vitamin K administration was not clearly established.

Dear Sir:

Concerning the letter of H. Cremer and

D. Hotzel (*Nutrition Reviews* 18, 63 (1960)), I would like to point out that the stimulatory effect of penicillin on thiamine biosynthesis in the intestinal tract has been reported (*J. Nutrition* 54, 461 (1954); 65, 161 (1958); *Brit. J. Nutrition* 9, 340 (1955); abstracts of meeting of American Chemical Society, Chicago 1953, p. 26A; *Fed. Proc.* 13, 476 (1954); 17, 483 (1958)).

While this increased amount of thiamine was found to be made available to the rat by coprophagy in the case of thiamine (*Fed. Proc.* 18, 550 (1959); *J. Nutrition* 69, 81 (1959)), in the case of pantothenic acid deficiency and antibiotic response, the increased response was found not to be related to coprophagy since the pantothenic acid formed was directly available to the animal (*J. Nutrition* 69, 81 (1959)).

B. CONNOR JOHNSON  
Urbana, Illinois

### Nitrogen Balance and Exchangeable Albumin Pool

Since the protein metabolism of the undernourished or postoperative patient is dependent upon many factors such as nutritional intake, infection or actual loss of protein from the body, one can obtain the best overall index as to what is actually occurring by applying as many different methods to the same problem as possible. Recently R. E. Madden (*Lancet* II, 939 (1959)) studied the nitrogen balance in three undernourished patients before and after seven days of refeeding with a high-protein liquid diet. Two of the patients had upper gastrointestinal malignancies and were severely cachectic and the third had carcinomatosis. In addition to carrying out nitrogen balance studies, Madden measured the total exchangeable albumin pool both before and after the seven days of refeeding by using radioactive iodinated albumin.

It was found that the three subjects had positive protein balances of 259 to 371 g. for

the seven-day refeeding period, indicating that they were synthesizing large amounts of protein during this period. However, no significant change resulted in the size of the albumin pool expressed in grams. The changes in the pool ranged from 9.2 g. to -6.5 g. In the two patients with malignant gastric disease the half-life of the albumin was 5.3 and 8.2 days, while the half-life of the albumin in the patient with carcinomatosis was 16 days.

It seems unlikely that the patients could accumulate between 371 and 259 g. of protein without any change in the serum albumin level, unless all the protein was used for tissue or muscle. This discrepancy might be explained by the loss of large amounts of the tracer in the bowel, a condition which has been reported to occur in patients originally believed to have hypercatabolic hypoproteinemia (*Nutrition Reviews* 17, 261 (1959)). This might also explain the short half-lives of the albumin noted in two of these individuals.

This investigator concludes that the total of exchangeable albumin pool measured in these three hypoproteinemic patients did not show significant increases in size after refeeding on a high-protein, high-calorie diet, in spite of positive protein balances of 40 to 50 g. per day. Such a study is of interest and should be repeated using polyvinylpyrrolidone tagged with radioactive iodine to show any possible losses of protein into the gut. Under such conditions it might be possible to seek out discrepancies between the two methods and arrive at a physiological explanation compatible with all procedures.

### Recent Books

- The Low Sodium, Fat-Controlled Cookbook.* By Alma Smith Payne and Dorothy Callahan. Published by Little Brown & Co., Boston, Mass. Pp. 429. Price \$4.75.
- The Merck Index of Chemicals and Drugs,* Seventh Edition. An encyclopedia for chemists, pharmacists, physicians and members of allied professions. Published by Merck & Co., Rahway, New Jersey. 1960. Pp. 1641.
- Modern Nutrition in Health and Disease.* Second Edition. Edited by Michael G. Wohl and Robert S. Goodhart. Lea & Febiger, Philadelphia, Pa. 1960. Pp. 1152. Price \$18.50.
- Foods Without Fads.* E. W. McHenry, University of Toronto. J. B. Lippincott Co., Philadelphia and New York. 1960. Pp. 159. Price \$3.50.
- Nutrition in Clinical Dentistry.* Abraham E. Nizel. W. B. Saunders Co., Philadelphia and London. 1960. Pp. 467. Price \$10.00.
- New Jersey State Diet Manual.* Project of Diet Therapy Section, New Jersey Dietetic Association, 1958-1959. Published by New Jersey State Department of Health. Pp. 84.
- Losses due to Agricultural Pests.* Summary of Conference of the Agricultural Board Committee on Agricultural Pests, November 1959. National Academy of Sciences-National Research Council. Pp. 20.
- The Measurement of Grassland Productivity.* Edited by Professor J. D. Ivins. Academic Press, Inc., New York. 1959. Pp. 212. Price \$7.00.
- Essentials of Healthier Living.* By Justus J. Schifferes, Director, Health Education Council. Published by John Wiley & Sons, Inc., New York, N. Y. Pp. 327. Price \$5.50.

## FACTOR 3, SELENIUM AND VITAMIN E

The trails of nutrition research have often taken unexpected turns. One such turn occurred in 1957, when it was discovered that the element selenium plays a part as an essential micronutrient. This finding was made when selenium was identified as an integral constituent of Factor 3 (K. Schwarz and C. M. Foltz, *J. Am. Chem. Soc.* **79**, 3292 (1957)). Factor 3, first described as an unknown agent protecting rats against dietary necrotic liver degeneration, has now been recognized to prevent a wide spectrum of deficiency diseases; for example, multiple necrosis in mice, muscular dystrophy and heart necrosis in mink, exudative diathesis in chicks and turkeys, stiff lamb disease and ill thrift in sheep, white muscle disease in calves, and liver dystrophy and muscle degeneration in pigs.

In some of these fatal diseases, Factor 3 has displayed a remarkable partnership with vitamin E and L-cystine. In fact, it was designated "Factor 3" to indicate that it was the third dietary agent found to be effective against liver necrosis. Meanwhile, the role of L-cystine has appeared in a new light; this sulfur amino acid owes its potency largely to a minute contamination with a trace of Factor 3-active selenium.

The Factor 3 problem started in 1939 as a side line to studies on bacterial growth factors. In order to investigate whether the growth factor H' was essential to animals, a rigorously purified casein was produced (casein VI). Factor H', isolated and identified as para-aminobenzoic acid (R. Kuhn and Schwarz, *Ber. Dtsch. Chem. Ges.* **74**, 1617 (1941)), was not essential for rats. Yet, most of the animals on casein VI diets died after slightly more than a month from a sudden attack of liver necrosis. Against this deficiency disease, some natural products such as wheat germ, wheat bran, and whey

were found to contain protective agents. Using prevention of liver necrosis as an assay, a study of these protective substances was begun. Through fractionation from wheat germs, tocopherol was identified as one of the main liver protecting agents (Schwarz, *Z. f. Physiol. Chem.* **281**, 109 (1944)).

Liver necrosis on deficient diets had been observed by many others, especially in rats on low protein rations. In retrospect, it seems that the diverse diets used in all these studies were low in sulfur amino acids, and deficient in vitamin E and Factor 3. An early publication was that by T. E. Weichselbaum (*Quart. J. Exp. Physiol.* **25**, 363 (1935)). He fed low cystine diets, observed "hemorrhages throughout the liver", and found that L-cystine prevented the damage. The protective effect of commercial L-cystine on liver necrosis was then more clearly established by F. S. Daft, W. H. Sebrell and R. D. Lillie (*Proc. Soc. Exp. Biol. Med.* **50**, 1 (1942)).

That a third factor, other than L-cystine and vitamin E, was involved in the prevention of the disease became apparent early in our studies, but conclusive proof for the factor was obtained only after 1949. In Europe, it has been found during World War II that certain yeasts could be used as sole source of protein in diets which induced necrotic liver degeneration, but in the United States workers were unable to duplicate these findings with American yeasts. Some laboratories resorted to imported British bakers' yeast to produce the disease, until an American grown *Torula* yeast was found to be useful for the purpose (Schwarz, *Proc. Soc. Exp. Biol. Med.* **77**, 818 (1951)). In the Section on Experimental Liver Diseases at Bethesda, a semisynthetic basal diet containing 30 per cent *Torula* yeast has been in use since then with consistent results. Con-

vincing proof for the existence of Factor 3 was obtained by supplementation of a few per cent of American brewers' yeast to this ration. The addition afforded complete protection. The same effect was elicited by small supplements of "vitamin-free" caseins, shown to contain Factor 3. The results indicated that liver necrosis was not caused by some hypothetical "necrogenic" influence; it simply constituted a deficiency of complex origin.

Comprehensive studies were carried out in the following years to determine the natural distribution of Factor 3, to devise suitable fractionation and isolation procedures for it, and to clarify its role in intermediary metabolism. Factor 3 was found to be widely but unevenly distributed in nature. Even in kidney, a potent natural source, it is present only in minute absolute amounts. Like many other micronutrients, Factor 3 is bound to its natural environment, apparently to protein, and cannot be extracted except after autolysis or hydrolysis. The factor is of low molecular weight, acidic, soluble in water and polar solvents like alcohol, but insoluble in lipid solvents.

In the course of the fractionation procedures, an enzymatic brewers' yeast autolysate, a digest of casein, and acid hydrolysates of dried, fat-extracted pork kidney were used as source materials. Sizeable batches of raw material had to be worked up, and several thousand rat assays with Factor 3 fractions were run before highly potent, microcrystalline, but still impure Factor 3 fractions could be obtained in milligram quantities. These displayed a characteristic, garlic-like odor, which suggested the presence of selenium.

Analyses of Factor 3 preparations of greatly divergent activities revealed that the biopotency was correlated to the selenium content. Then it was realized that rats can utilize known compounds of selenium very effectively. It would be erroneous, however, to equate Factor 3 simply with selenium. Large differences in potency exist

between different selenium compounds, and, per atom of selenium, Factor 3 is more potent than any other compound tested thus far.

It has occurred repeatedly in the field of nutrition that, with the identification of a new dietary agent, several other seemingly distant questions found their solution. This has also been the case with Factor 3. In 1955, M. L. Scott and collaborators adapted the *Torula* yeast ration to the production of vitamin E-deficiency in chicks. The diet caused growth failure, exudative diathesis, and death (Scott *et al.*, *J. Nutrition* **56**, 387 (1955)). It was ascertained during the same year that Factor 3 effectively prevented this disease in chicks, just as it prevented dietary liver necrosis in the rat. Scott *et al.*, at Cornell, as well as J. G. Bieri and G. M. Briggs at Bethesda, assayed our Factor 3 concentrates from kidney, brewers' yeast and casein. Between both species, a close parallelism was found in the effectiveness of Factor 3 preparations, some crude, and some several hundred-fold concentrated. On the basis of these results, a group at Lederle Laboratories started to use the chick assay and the *Torula* yeast diet for an investigation into the nature of Factor 3.

After the discovery of selenium in Factor 3 was made in March, 1957, in our purification project using the rat assay, a cooperative study was arranged between Cornell and Bethesda to test the potency of selenium compounds in chicks. Selenite and selenocystathionine were highly potent; elementary selenium protected at very high levels (Schwarz, J. G. Bieri, G. M. Briggs and Scott, *Proc. Soc. Exp. Biol. Med.* **95**, 621 (1957)). The Lederle group had observed that alkaline but not acidic ashes of crude Factor 3 fractions retained approximately 20 per cent of the activity. They independently described the effect of selenite against exudative diathesis (E. L. Patterson, R. Milstrey and E. L. R. Stokstad, *Ibid.* **95**, 617 (1957)).

Selenium is one of the rarer elements, with properties very similar to those of sulfur. The two often occur together, and there is a



rather complete analogy between the inorganic and organic compounds of both elements. Selenium analogues of the sulfur amino acids have been found in biological materials, such as proteins of seleniferous plants, and can be synthesized. With respect to biopotency against liver necrosis in rats, selenium compounds can be divided into three main categories (Schwarz and C. M. Foltz, *J. Biol. Chem.* **233**, 245 (1958)). Elementary selenium and certain inorganic and organic compounds are practically inactive. A second group affords protection at levels supplying a few micrograms of the element per 100 g. of diet. Their 50 per cent effective dose ( $ED_{50}$ ) is about 2 to 3 micrograms per cent selenium (0.02 to 0.03 p.p.m.). Most inorganic selenium compounds, such as selenate and selenite, many organic selenium compounds, and also the selenium analogues of cystine, cystathionine and methionine belong to this category. It appears that the rat and the chick can utilize selenium from all these sources with equal efficiency.

The third category, comprising substances of higher biopotency, is small. Thus far only four synthetic organic compounds were found to be more active than selenite. None of them, however, reaches the biopotency of Factor 3. The latter has an  $ED_{50}$  of about 0.7 micrograms per cent selenium, as established by standardization of various Factor 3 preparations in the animal assay and determination of their selenium contents by radioactivation analysis, colorimetric assay and micro-analytical methods. Radioactivation analysis is the method of choice; it has a sensitivity of 0.01 micrograms selenium per gram of material. The method consists of the production of radioactive isotopes by neutron bombardment and subsequent determination of the element in question by carrier analysis. A daily dose of 0.1 microgram of Factor 3-selenium fully protects rats against death. It also enhances growth significantly. This requirement is in accordance with the amounts of selenium

found in natural nutrients, which are of the order of 0.05 to 10 micrograms per gram of dry matter.

Selenium estimation, however, does not result in an estimate of Factor 3 activity, since some nutrients contain the element in a form which is biologically inert. At the moment the animal assay is the only feasible method for the determination of the Factor 3 content of dietary ingredients.

One atom of selenium, in form of Factor 3, affords as much protection as 700 to 1000 molecules of vitamin E in preventing dietary liver necrosis in the rat, multiple necrotic degeneration in the mouse, and exudative diathesis in the chick. L-Cystine, in turn, possesses only a very small fraction of the potency of tocopherol; to elicit 50 per cent protection, about 0.4 per cent must be added to the diet. The biopotency of Factor 3 is so high that a trace contamination of one atom of Factor 3-selenium among 355,000 atoms of sulfur could account for the biological potency of the sulfur amino acid. This amount is indeed found in commercial L-cystine, which is manufactured from horn, hoofs and hair and contains up to 2 micrograms of selenium per gram. Procedures which remove the selenium from the cystine also eliminate the biopotency (Schwarz, J. A. Stesney and Foltz, *Metabolism* **8**, 88 (1959)).

Methionine and cystine, when clearly free from traces of selenium, do not prevent liver necrosis. However, they delay its onset when supplied at levels exceeding the requirement for growth. The delay is the result of a sparing effect on the requirement for vitamin E. The sulfur amino acids reduce the vitamin E requirement to only one tenth of the level normally necessary for protection. Thus they exert a modulating effect on the condition, but in essence dietary liver necrosis is the result of the simultaneous lack of Factor 3-selenium and vitamin E.

While the lack of Factor 3 alone, or that of vitamin E by itself, produces often relatively mild chronic diseases, the simul-

taneous deficiency of both leads invariably to acute, impressive tissue damage and to death. The pathological signs of what in the past was thought to be vitamin E-deficiency range from mild afflictions, such as resorption sterility or testicular atrophy, to fatal changes in liver, kidney, heart, muscles, lungs, pancreas, brain and spinal cord. A number of these diseases, hitherto attributed solely to vitamin E, are of dual origin. The presence or absence of Factor 3-active selenium determines the fate of an animal on a vitamin E-deficient diet.

Regardless of this interrelationship, selenium neither spares vitamin E, nor does it substitute for it. It is an essential dietary constituent in its own right. In rats provided with tocopherol, Factor 3-deficiency causes a distinct disease entity comprising lack of growth, muscular wasting, adrenal atrophy and pancreatic dystrophy. In the chick, Factor 3-active selenium has a growth effect independent of vitamin E. And in ruminants, selenium begins to play an important practical role in "stiff lamb disease", and in white muscle disease in calves (O. H. Muth, J. E. Oldfield, L. F. Remmert and J. R. Schubert, *Science* **128**, 1090 (1958); J. F. Proctor, D. E. Hogue and R. C. Warner, *J. Animal Sci.* **17**, 1183 (1958)).

These diseases, consisting of muscular dystrophy, calcification, and heart muscle degeneration, are enzootic in the north-western United States, New Zealand, Scotland, Sweden, Finland, etc. In some areas, they are affected only little by vitamin E. A single dose of 1 mg. of selenite selenium greatly increases the number of live-born lambs, prevents congenital white muscle disease, as well as field outbreaks of white muscle disease in lambs, and eliminates post-weaning "unthriftiness", probably the most important cause of economic loss in this industry in New Zealand (C. Drake, A. B. Grant and W. J. Hartley, *New Zealand Vet. J.* **8**, 4 (1960)).

In principle, then, one must separate clearly three groups of diseases: those caused

purely by vitamin E deficiency, which are not influenced by Factor 3-selenium even in large excess (resorption sterility in rats, encephalomalacia in chicks); those caused purely by Factor 3-selenium deficiency, which are not affected by vitamin E; and those caused by simultaneous lack of both factors. To the last category belong dietary liver necrosis in the rat, multiple necrosis in the mouse, heart and peripheral muscular dystrophy in the mink, liver necrosis and muscular dystrophy in the pig, and exudative diathesis in chicks and turkeys.

It is common medical knowledge that a specific damage, for example an infection, shows different sites of predilection in different species. The same applies to deficiency diseases. Necrotic liver degeneration is only a special instance of a widespread injury involving many other tissues as well. The liver is the site of predilection in the rat. In other animals, damage to other organs is more predominant. The heart, for instance, is involved in mice, mink and sheep, the peripheral muscles degenerate in most species, and kidneys, pancreas, lungs, adrenals, etc., are more or less severely damaged in most mammals, while exudative diathesis, probably due to hypoproteinemia, prevails in birds.

The involvement of so many tissues may indicate that a function of fundamental importance to metabolism breaks down. Studies with liver slices from rats on the necrosis-producing diet showed that a metabolic defect, respiratory decline, is present one to two weeks before actual necrosis occurs. Such liver slices cannot maintain normal oxygen consumption. Addition of vitamin E to the diet, or the intravenous injection of the vitamin ten to 30 minutes prior to extirpation of the liver will completely prevent the breakdown. Factor 3-selenium affects respiratory decline independently, but to a lesser degree. It seems that the impairment which leads to liver necrosis involves energy metabolism.

A working hypothesis to explain the

synergistic partnership of vitamin E and selenium postulates that the two have independent catalytic effects in alternate pathways of metabolism (Schwarz, *Liver Function*, p. 509. *Am. Inst. Biol. Sciences, Washington, D. C. (1958)*). This would explain why the absence of both is necessary for the development of most of these injuries.

In the past, selenium has received a great deal of attention for its poisonous properties, and hardly any for beneficial effects. Thus S. F. Trelease and O. A. Beath have stated in their outstanding review on selenium poisoning that "as far as we know it is toxic, but never beneficial to animals or man" (Trelease and Beath, *Selenium*, New York (1949)). The toxicity of selenium compounds varies greatly with their chemical constitution, and it is dependent on the nutritional status of the animal. The minimum chronic toxic dose of selenite-selenium is approximately 300 to 400 micrograms per 100 g. of diet (3 to 4 p.p.m.). If this level is compared to that which protects against Factor 3-deficiency, we see that selenium is relatively

not more toxic than other dietary constituents. The ratio between effective dose and toxic dose is of the order of 1 to 100, which compares favorably with the therapeutic indices of amino acids, salts, and almost all other important nutrients.

The discovery of Factor 3 and the detection of the biological role of selenium serve to illustrate the point that there may still be new essential dietary agents left to be explored through animal experimentation in conjunction with modern methods of biochemistry. One may be tempted to think that a factor with protective qualities against serious lesions in so many species may play a role in man. Exacting and critical studies on human diseases are needed in areas indicated by animal experiments in order to substantiate this conclusion.

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### PROTECTIVE EFFECT OF MILK AGAINST BONE STRONTIUM-90 ACCUMULATION

*Although the level of strontium-90 in milk has been gradually increasing, the high calcium content of milk makes it one of our best protective foods for the prevention of strontium-90 accumulation in humans.*

Since milk and milk products make up a large part of the diet of Americans and especially the diet of children, the reports released by the U. S. Public Health Service of gradually increasing levels of strontium-90 in milk have led to increasing concern on the part of both professional and lay persons. Although there has been interest and research into the possibility of removing strontium-90 and other radionuclides from milk and into the possibilities of milk substitutes, a consideration of the significance of strontium-90 in milk and milk products in

terms of human accumulation of this source of radiation seems most opportune.

B. L. Larsen (*J. Dairy Sci.* **43**, 1 (1960)) has reviewed the currently available information on strontium-90 in milk and the effect of milk on strontium-90 accumulation in the human population. His survey gives strong support to the claim that milk is still our most satisfactory protective food, whether in terms of supplying needed nutrients or in terms of preventing strontium-90 accumulation in the bones of our population.

Strontium-90 originates primarily in fallout, and the factors which influence fallout are of great concern to all of us. The three most important factors in fallout are (1) the size and location of nuclear explosions made to test nuclear weapons, (2) the winds of the various latitudes and (3) the annual rainfall in any given area. E. A. Martell (*Science* 129, 1197 (1959)) has presented data indicating that the amount of fallout at the forty-fifth latitude north has been approximately five times that of fallout at the forty-fifth latitude south and 15 times that at the equator. M. Eisenbud, in considering this same problem (*Ibid.* 130, 76 (1959)), has indicated that the bulk of the fallout lies between the fortieth and fiftieth latitude, both north and south, but that the fallout in the north latitudes is three times that in the south.

The increasing rapidity with which strontium-90 fallout has developed has led to consideration of the strontium-90 present in rainfall. At present it appears that cistern water may be giving more strontium-90 than is secured from foods, and recent data indicate that cistern water is now averaging from 3 to 5 micro microcuries of strontium-90 per liter.

With the accumulation of data indicating a much higher rate of fallout in the northern latitudes, it is of particular interest that J. L. Kulp, A. R. Schulert and E. J. Hodges (*Science* 129, 1249 (1959)) have reported that accumulations of strontium-90 in human bones are similar in both the northern and southern hemispheres. That there has been a continuing increase over the past ten years in the strontium units (a strontium unit, "S.U.", is one micro microcurie of strontium-90 per gram of calcium) is revealed by their data, which shows an increase from a value of approximately 0.12 S.U. to approximately 0.19 S.U. in human bones.

A number of factors appear to influence this increase in strontium-90 in human bones. In the southern hemisphere, vegeta-

bles and cereals supply most of the calcium in the diet as opposed to milk in the northern hemisphere. Moreover, there is a high rainfall in tropical regions and a relatively low calcium availability in tropical soils. Finally, there is a global flow of food products, such as wheat and dried milk, which tends to equalize the types of diet and the levels of strontium-90 in the diet in both hemispheres. However, it should be pointed out that the highest levels of bone calcium were found in those people on rice diets in the southern hemisphere. Thus, despite the apparent equalization of strontium-90 in the bones of the human population, it appears probable that the populations receiving dietary calcium from milk and milk products are accumulating relatively lower levels of strontium-90 in their bones than those consuming primarily vegetable and cereal products.

As of January 1958, adults had an average bone strontium-90 accumulation of 0.19 S.U. while children had approximately five times this amount. It is estimated that, with the present strontium-90 burden of the atmosphere and soil, a maximum value or equilibrium in children will be reached in 1966 with approximately 4.0 S.U. in the bones.

These values must be viewed against a background of what is considered a hazard. The National Committee on Radiation Protection in reviewing the permissible burdens of strontium-90, cesium-137 and iodine-131 for workers in atomic plants have set the permissible strontium-90 bone level as 2 microcuries and the cesium-137 level in muscle as 50 microcuries. On the other hand, the National Committee on Radiation Protection has arbitrarily set the levels for populations in the vicinity of atomic installations at one-tenth of these levels. By comparison then, workers in atomic plants have a permissible burden of 2000 S.U. whereas the neighbors to these plants have a permissible level of 200 S.U. The permissible level for the population as a whole has been set at 100 S.U., compared with 4 S.U. as the predicted level for children in 1966

(*National Bureau of Standards Handbook 69* (1959)).

The reports on strontium-90 in milk indicate a steadily increasing value as more and more fallout returns to the soil from the atmosphere, yet in 1958 at the cessation of nuclear testing, values for milk from many cities around the country indicated average values ranging from 2 to 16 S.U. per liter.

Although milk has been considered in greater detail, increasing attention has been given more recently to the strontium-90 accumulation in cereals, vegetables, fruits and other items of the diet. The vegetable and cereal strontium-90 values have led to a realization that, especially in the adult diet, these products may actually be supplying from five to 20 times the level of strontium-90 supplied in the diet by milk. For example, the 1958 wheat crop showed from 64 to 330 S.U., and in the August 14, 1959 issue of the *Washington Post* there was a report that the Food and Drug Administration had found strontium-90 in alfalfa at levels ranging from 136 to 806 micro microcuries of strontium-90 per kilogram.

These values, which have been reported variously as micro microcuries of strontium-90 per kilogram and as strontium units (which take into consideration the calcium level), may be misleading unless the widely varying calcium content of vegetable products is recognized.

The reporting of strontium-90 as micro microcuries, rather than as strontium units, places major emphasis upon the level of strontium-90 in the diet rather than on the strontium-90 that will accumulate in the bones. Milk may contain ten times as much calcium as potatoes, but, if milk and potatoes contain the same strontium-90 level, a diet of potatoes will give a bone deposit of strontium-90 that will be five to ten times that from a diet of milk.

The only satisfactory way to evaluate a diet must be in terms of both calcium content and strontium-90 level. Because most foods contain less than 1 g. of calcium per

kilogram, the micro microcuries of strontium-90 per kilogram will be lower than the strontium units. This may lead to a tendency to eliminate those foods with highest strontium-90 content, not recognizing that if calcium levels are thus ignored, the calcium level of the diet will be reduced and deposition of strontium-90 will increase.

On the basis of strontium units, calculated from a report of the levels found in dietary items in those areas influenced by uranium refinery waste (*U. S. Public Health Service Report. Survey of Interstate Pollution of the Animus River* (1959)), it was noted that milk contained 5 S.U., cabbage 210 to 682 S.U., potatoes 37 to 50 S.U., peas 1137 S.U., sweet corn 34 to 200 S.U., apples 110 S.U., lettuce 86 to 4420 S.U. Interestingly enough, alfalfa, wheat and oats in these same regions contain less than 10 S.U.

All of these results pose very serious problems in the effort to determine the effects of long-term, low-level radiation on humans. If the present accumulation is negligible, then there is no problem. Certainly current evidence is that the present and projected levels of accumulation are minor compared to natural sources of radiation such as potassium-40 and carbon-14, or compared to the irradiation from medical practice. However, until we have more definite data, the only reasonable procedure is to keep strontium-90 accumulation to a minimum.

Efforts to reduce strontium-90 accumulation in the bone must take into consideration the fact that the assimilation and hence the incorporation into bone of strontium-90 from milk is decreased by an increase in the calcium level of the milk, or of the diet as a whole. A source of calcium which did not contain strontium-90, such as mineral sources of calcium, would then have a real advantage. Reducing the strontium-90 which comes from other foods should also be considered, since it appears that there is less discrimination against strontium-90 in low-calcium vegetable and cereal items. It certainly is probable that as the strontium-90

level of vegetable items of the diet increases, milk will provide the greatest protective action because of the discrimination by the body of the cow and the human body against strontium in favor of calcium.

This discrimination may be even more important if results recently reported by A. E. Sobel, S. Nobel and P. A. Laurence (*Chem. Eng. News* 37, 42 (1959)) are correct. Their results indicate that strontium-90 may not enter into the crystalline structure of bone and, therefore, would be subject to exchange with calcium, favored by high calcium- and vitamin D-containing diets. This would explain the much more rapid removal of strontium than of calcium from bone observed by others (A. R. Schulert *et al.*, *Int. J. Applied Radiation & Isotopes* 4, 144 (1959)).

The data of Larsen emphasize the many problems to be considered in any evaluation of the role of milk in strontium-90 accumulation in man. More particularly, the data emphasize that milk is still the best protective item in the human diet. The need for more knowledge about the strontium-90 content of other items in the diet and, particularly, about the effects of diet variation upon strontium-90 accumulation certainly appears crucial. However, in the light of this review of the role of milk, it appears that because of its protective action, a more satisfactory reduction in terms of strontium-90 accumulation in bones may be obtained by doubling the calcium level of the diet rather than by removing as much as 50 per cent of the strontium-90 from the milk.

### MAGNESIUM BALANCE IN ALCOHOLICS

*Using metabolic balance techniques, alcoholic patients were found to retain more magnesium than non-alcoholic hospital patients used as controls. This was interpreted as indicating that the alcoholic patients were deficient in magnesium.*

While magnesium has been known for some time to be an indispensable nutrient, its daily requirement for man is not known. Dogs and rats fed diets grossly deficient in magnesium develop a clinical picture which includes vasodilation, cardiac arrhythmia, exaggerated response to external stimuli, spasms and convulsions. If the deficiency is not relieved then death ensues. In the case of man, magnesium deficiency has been associated with tremors of the extremities, choreiform and athetoid movements of all muscles, convulsions, sweating and degrees of mental clouding which may progress to delirium and death (*Nutrition Reviews* 18, 72, 101 (1960)). Since magnesium is widely distributed in most foods, an individual eating a general mixed diet does not develop a deficiency of this substance.

Chronic alcoholics as a group possess a wide spectrum of nutritional deficiencies because they obtain their caloric needs

mainly from drinks which are deficient in most nutritional substances except alcohol, water and carbohydrate.

Recently, R. J. McCollister, E. B. Flink and R. P. Doe (*J. Lab. Clin. Med.* 55, 98 (1960)) studied the status of magnesium in a group of 11 individuals. Using the metabolic balance technique, they first investigated the level of magnesium in a group of seven non-alcoholic men, hospitalized for treatment of peptic ulcer, who served as controls. Five of these men, fed diets consisting of milk or half milk and half cream plus supplementary foods such as toast or eggs, were studied for periods ranging from four to eight days.

Their magnesium intake varied from 87.9 to 154 mEq per period (17 to 24 mEq per day) with an average value of 113.9 mEq, while their urinary output varied from 17 to 104 mEq per period with the high and low values corresponding with the high and low



intakes. Their fecal excretion was quite constant, ranging between 59 and 82 mEq per period. The magnesium balance for these individuals varied from +17 to -27 mEq per period, equivalent to +3.4 to -4.5 mEq per day.

Two men of the same type were maintained on a similar diet and, in addition, were given magnesium sulfate intramuscularly at the rate of 8 or 16 mEq per day for a six-day period. The total magnesium intake for these two patients was 123 and 161 mEq per period (20 to 27 mEq per day). Their urinary output was increased above those who did not receive the injections of magnesium and averaged 181.8 mEq. per six-day period. Both individuals showed a positive magnesium balance of 4.8 and 10.0 mEq per period (1.3 and 1.8 mEq per day).

Six alcoholic patients were treated with the diet alone for periods of six to ten days. They had an average magnesium intake of 208 mEq per period (17 to 31 mEq per day). Under these conditions their magnesium balance varied from +39 to +151 mEq per period (from 6.0 to 16.8 mEq per day). Five additional alcoholic patients treated with the diet plus magnesium had intakes ranging from 97 to 350 mEq per period (all periods were six days except for an eight-day period for the last individual). This intake amounted to 16 to 43 mEq per day. These patients had positive magnesium balances of 35 to 170 mEq per period, ranging from 6 to 21 mEq per day, with the largest balance in the patient who received the largest intake.

These investigators concluded that, since the alcoholic patients on the magnesium refeeding program retained considerably more magnesium than did the hospital patients used as controls, the magnesium stores of the alcoholic patients were grossly depleted. Also they noted that there was little change in the serum magnesium levels even after the patient showed a very good positive magnesium balance.

While in general these investigators reported that the alcoholic patients showed good positive magnesium balances after magnesium administration, it should be noted that, even before they were given intramuscular magnesium, the alcoholic patients had a much larger intake of the element than did the hospital control patients (208 mEq compared to 113 mEq). Thus one is not sure whether the non-alcoholic and supposedly non-depleted patient might show a much better positive magnesium balance had he received a greater magnesium intake.

Since magnesium deficiency also has been reported to occur in patients maintained on magnesium-free parenteral fluids (glucose or sodium chloride but not protein hydrolysates since they contain magnesium), magnesium deficiency may also occur in patients ill with diarrhea, vomiting, many types of malnutrition and some osteolytic bone lesions, as well as in patients who are taking very little food orally. Thus the possibility that magnesium deficiency occurs in a large number of patients may be quite real. More investigations in this field may lead to better care.

### PROTEIN MALNUTRITION IN SOUTH INDIA

*A combination of poverty, ignorance and disease has resulted in a general deficiency in calories and a specific deficiency in protein for most of the children of South India.*

From 1955 to 1957 the World Health Organization and the Indian Council of Medical Research sponsored a survey of protein malnutrition in the South India states of Kerala, Madras, Mysore and And-

hra Pradesh. Some of the preliminary results obtained in this survey have already been published (*Nutrition Reviews* 15, 169 (1956); S. G. Srikantia, S. Sriramachari and C. Gopalan, *Ind. J. Med. Res.* 46, 121 (1958);

Gopalan, *Ibid.* 46, 317 (1958)). A more complete summary of the results has recently appeared (K. S. Rao, M. C. Swaminathan, S. Swarup and V. N. Patwardhan, *Bull. World Health Org.* 20, 603 (1959)).

The region considered in the study covers 248,130 square miles with a population of 94.8 millions, ranging from 261 per square mile in Mysore to 908 in Kerala. Over 80 per cent of the population is agricultural, less than half of which till their own land. The principal foodstuffs raised in the region are cereals (mainly rice and millets) with smaller amounts of pulses, tapioca, milk, fish and vegetable oils. The per capita production of calories is 1842 per day including 45 g. of protein.

The 4536 children studied were divided about equally between urban and rural areas, but almost all were from the less-favored social groups. More than half the families had incomes of less than 50 rupees per month (1 rupee is about 21 cents) and less than 1 per cent of them had incomes greater than 150 rupees per month. Their living conditions, whether in city or country, were inadequate and unsanitary.

The main foods consumed by most of the families studied consisted largely of cooked cereals (largely rice or millets) and a spiced soup containing pulses and vegetables whenever available. Meat was available very infrequently and fish and milk, when produced, were largely sold in the cities so that few of these protein-rich foods were available for consumption at their source.

The children themselves had few of the advantages of modern medical practice or even of the most rudimentary cleanliness and hygiene. Not only poverty, but also ignorance and tradition were responsible for these conditions, which were so general that disease was commonplace, rarely treated effectively and probably responsible in part for the nutritional disorders which were the subjects of this study.

Weaning customs differed in the several states, but in general, about 50 per cent of

the babies were weaned at 18 months and 80 per cent at 24 months. Except in Hyderabad (Andhra Pradesh), most of the babies received some supplementary food at one year, usually cereals, rarely animal milk (except in Mysore). Although the protein content of breast milk of Indian mothers was found to be in the normal range, the protein and caloric intakes of children between six and 36 months were considerably below the FAO recommendations, and it is in this period that kwashiorkor is most frequently found. Up until six months the mothers' milk was apparently adequate, while after three years of age the children consumed an average diet.

Clinical examination revealed that most of the symptoms associated with protein malnutrition were common. One quarter of the children examined had had recurrent diarrhea while 2 to 5 per cent had had edema. Over 60 per cent had dry inelastic skin, if not the typical dermatosis of kwashiorkor. Sparseness and depigmentation of the hair, "moon face" and hepatomegaly were common in all regions. As many as 38 cases of frank kwashiorkor were found in the homes, although most cases had been removed to the hospital (where this disease accounts for 5 to 16 per cent of pediatric admissions). However, it is interesting that the average serum hemoglobin and serum protein in children with these symptoms were no lower than in the children without apparent symptoms. Most of the children appeared to have some degree of emaciation, and heights and weights of the children examined were well below the lowest percentiles for American children of the same age groups. It was thus apparent that the Indian children were suffering not only from a deficiency of protein but also of calories and, probably, of vitamins.

To bring the problem into focus, the authors state that by the most conservative estimates there are at least 120,000 cases of kwashiorkor in the region under study at any one time, while millions are suffering

from milder forms of protein malnutrition. They list three major causes for this sad state of affairs: poverty, with most families so poor that their entire incomes could not provide adequate nourishment; ignorance, which prevents the use of protein-rich foods for children even when available and especially when needed; infection and infestations, which often trigger the attacks of kwashiorkor and which aggravate an always marginal state of nutrition.

The solution to these problems is not going to be simple or rapid. However, research of the type reported by the present

authors demonstrates that adequate protein nutrition in India can be achieved by supplementation of the usual high-cereal diet with high-protein protective vegetables such as the pulses and, where possible, with animal products such as milk and fish. The importance of this solution is that no new or expensive foods need be introduced to an unwilling public. The main problem, then, appears to be one of education, to convince the people that nutritional health is desirable and to show them how to achieve it and, at the same time, improve their economic status.

### RECOMMENDED DIETARY ALLOWANCES

*The newly revised allowances are discussed by appropriate representatives in terms of their uses and applicability to the general population, the armed services and the food industry.*

The Recommended Dietary Allowances were first published by the Food and Nutrition Board of the National Research Council in 1943. The latest revision (*National Academy of Sciences-National Research Council Publication No. 589, Washington, D.C. (1958)*) represents the fourth since its original presentation. Recently, R. E. Shank has discussed the most significant changes introduced in this new edition (*Am. J. Pub. Health 49, 1001 (1959)*).

Prior to the establishment of Recommended Dietary Allowances, the usual standards met minimal needs. The allowances, however, sought higher objectives, being designed to maintain good nutrition in healthy persons in the United States under current conditions and to cover nearly all variations of requirements for nutrients in the population at large. They are, however, not considered adequate to meet the additional requirements deriving from disease or for nutrient repletion, but are more generous than is often practicable when large groups must be fed under conditions of limited food supply or economic stringency.

It was with the foregoing in mind that the following changes were made. The first of these relates to "reference" individuals. With the previous (1953) revision, the reference individuals had been changed to correspond with conditions which were specified by the Food and Agriculture Organization but which did not correspond to conditions prevailing in the United States. For instance, the weight of the reference man was 65 kg., whereas the average adult man, aged 25, is approximately 70 kg. in this country. Moreover, the FAO specified a mean external temperature of 10°C, whereas the average temperature under which most Americans live seems closer to 20°C. Accordingly, the reference man was changed to 25 years of age, 69 inches tall, weighing 70 kg. (154 pounds), living at a mean external temperature of 20°C and engaged in moderate physical activity. Similar changes were made for the reference woman.

The new revision also contains tables providing calorie allowances for persons of varied size and age. Available data substantiate the decreases in caloric require-

ments with advancing age. Accordingly, calorie allowances given for age 45 are now 6 per cent less and for age 65 are 21 per cent less than for age 25.

Estimates for calorie allowances were also modified with respect to climate. A 5 per cent decrease for each increase in temperature of 10 degrees was retained, but an allowance of only 3 per cent is given for each 10 degree decrease in external temperature. This change is based on the evidence that persons usually protect themselves from the cold by use of warm clothing and by living and working in heated buildings.

It is recognized that people engaged in heavy labor will have enhanced food requirements but that this requirement seldom need be increased more than 25 per cent above the standard allowance. On the contrary, the most important problem is thought to be the large proportion of persons who expend very little physical energy. Fortunately, according to R. E. Shank, there is renewed interest in assessing energy expenditures in common activities and employment. He suggests that perhaps a later revision of the Recommended Dietary Allowances will be able to offer more satisfactory modifications of the caloric requirements of physical activity.

The most time and effort was devoted by the Board to the consideration of protein allowances, and a new approach was sought in the light of world-wide research interest in protein requirements. However, the Board retained the approach to protein allowances utilized in earlier editions as well as the long-accepted value of 1 g. protein per kilogram of desirable weight. It was agreed that this allowance is not excessive and probably includes a reasonable margin of safety to meet variations in the consumer protein quality. A provision is made for an additional intake of 20 g. protein per day above the basal allowance during the last half of pregnancy and 40 g. per day for lactating women. No allowance was stated for infants during the first months of life because of the insufficiency of data. This deficiency of data

should serve as an inducement for heightening research efforts in this area.

Despite evidence of a wide variation in calcium requirements, as observed in balance studies, the allowance of 800 mg. of calcium per day for adults (excluding pregnancy and lactation) was continued. Iron allowances were revised downward from 12 mg. iron per day to 10 for the adult male, as compared to 12 mg. for the adult female. This recognizes the lower rate of iron loss in the male as compared to the female during the years of menstruation.

Vitamin allowances were little changed and any alterations were based primarily on the changes introduced in the size of the reference man and woman. The major exception was that of the niacin allowance which was re-evaluated in terms of the quantity of the preformed vitamin available in mixed diets plus that derived from its precursor, tryptophan.

Dr. Shank emphasizes that the allowances were planned for healthy, moderately active persons. The presence of acute or chronic illness may modify requirements markedly. In order to serve as a guide for planning adequate diets for population groups, allowances must be weighted not only in terms of distributions of ages, sexes and activity categories but also for losses due to storage, cooking and serving.

#### *RDA in Military Nutrition*

One of the most logical applications of the Recommended Dietary Allowances is in the maintenance of the military. T. E. Friedemann, H. F. Kraybill and C. F. Consolazio (*Am. J. Pub. Health* 49, 1006 (1959)) discuss this subject in terms of (1) the Army's basic dietary standard and (2) the soldier's actual nutrient intake. They point out that the Quartermaster General is responsible for the choice of foods, the formulation of menus and the administration of the feeding program in the Army. Since July 1941, the procurement and serving of food has been prescribed in a Master Menu published six months in advance and used throughout the

Army. This is based on the Recommended Dietary Allowances as modified by the special requirements of troops under various operational conditions.

The Army basic standard sets the minimum intake of the physically active soldier in a temperate climate as: 3600 calories, 100 g. protein, 700 mg. calcium, 5000 i.u. vitamin A, 75 mg. vitamin C, 1.7 mg. thiamine, 2.0 mg. riboflavin and 16 mg. niacin. During 1957 the average edible nutrients provided by the Master Menu were actually approximately 4195 calories, 131 g. protein, 199 g. fat and 470 g. carbohydrate. Vitamins and minerals were provided well above the recommended intake.

In surveys conducted in four training camps it was found that the average of nutrients consumed in the mess and from other sources was 4265 calories, 131 g. protein, 201 g. fat and 484 g. carbohydrate. The average caloric requirement for maintenance of weight in very active young soldiers was 4066 calories, while the average for moderately active soldiers was 3175 calories.

Of interest is the high fat content of the Army diet. Assuming that 1 g. of fat yields 9 calories of metabolizable energy, the 199 g. of fat in the 1957 menu furnished 43 per cent of the total daily calories of the ration. This must be considered high, but it is pointed out that it is within the range of fat-calorie intake of the high-income civilian groups in this country.

#### *RDA in the Food Industry*

According to H. E. O. Heineman and E. Bennett (*Am. J. Pub. Health* 49, 1013

(1959)), the Recommended Dietary Allowances have had a considerable influence on the food industry, awakening it to the desirability of staff personnel scientifically trained in nutrition and allied fields. This personnel has, for the most part, upgraded the standards by which the food industry operates. The Recommended Dietary Allowances are a useful guide for the food industry, and have spurred research to seek crops of highest nutrient quality, to develop better processing methods, and to find packaging materials and marketing systems which will maintain original nutrient values until consumption. They have helped establish the level of fortification of some of our foods, such as vitamin D-enriched milk and enriched bread and flour. They have also functioned as a barrier against low and useless additions of nutrients, although credit must also be given to the control exercised by the Food and Drug Administration.

The authors believe that the Recommended Dietary Allowances can act as an important checkrein despite intense competition in the food industry. Emanating from an unbiased group of scientists, they are a carefully controlled compilation constantly reviewed in the light of new research. Thus they can help the food industry maintain stability and balance in the midst of new, confusing and sometimes conflicting research data on specific nutrients and nutrient needs. It is to be hoped that the Recommended Dietary Allowances will be even more generously and wisely used by the food industry in the future.

### EFFECT ON SERUM CHOLESTEROL LEVELS OF REPLACING MILK FAT BY SOYA BEAN OIL

*By replacing butterfat with an emulsified soya bean oil and using a more unsaturated margarine in the diet, it was possible to reduce the serum cholesterol level in a series of patients.*

A great deal of activity has occurred in recent years investigating the possible relationship between dietary fats and blood

lipids with particular emphasis on cholesterol, and also the possible relationship between blood lipids and atherosclerosis.

Earlier issues of this journal have discussed the problem in great detail (*Nutrition Reviews* 15, 1 (1957); 16, 42 (1958); 17, 291 (1959)). Since the dietary consumption of fats, particularly in the form of butter and milk, is high in Finland and also elevated serum cholesterol values and atherosclerosis are common, this problem may be of great practical significance in that country.

Recently, O. Turpeinen and associates (*Lancet* I, 196, (1960)) studied the effect of replacing milk fat by emulsified soya bean oil in the diet of patients in two different mental institutions. The subjects were maintained on diets similar in calorie, protein and total fat content, except that patients in hospital "E," in place of butter or whole milk, were given margarine and skim milk supplemented with a 3.5 per cent emulsion of soya bean oil. The margarine used was a special type, considerably more unsaturated than the brands commonly on the market. Thus, although the patients in hospital "E" received a diet with a fat content similar to the control patients in hospital "C", the unsaturated fatty acid content of their diet was greatly increased and, conversely, the saturated fatty acid level decreased. The group in hospital "E" consisted of 117 patients with an average age of 49 years, and the control group in hospital "C" consisted of 53 patients with an average age of 48 years.

Before the start of the diet, the patients in hospital "E" had a mean serum chole-

sterol content of 235.7 mg. per cent, while after six months on the diet high in unsaturated fatty acids, the average blood cholesterol level had decreased to 215.3 mg. per cent. The difference between the two cholesterol levels was statistically significant ( $P < 0.001$ ). During the same period, the average serum cholesterol value of the control patients in hospital "C" rose from an initial value of 252.7 mg. per cent to a final value of 262.7, although in this case the difference between the two serum cholesterol levels was not statistically significant. The decrease in the blood cholesterol in the patients maintained on the diet in which the milk fat was replaced by soya bean oil was greatest for those with the highest initial cholesterol level.

While there is no doubt that there was a statistically significant decrease in the serum cholesterol level of patients maintained for six months on a diet in which the only change was the replacement of certain animal fats by a more unsaturated vegetable fat, the study has not yet proceeded long enough to determine whether or not there is any influence on the frequency of coronary artery disease and other atherosclerotic manifestations along with the decrease in cholesterol level. It also should be noted that the dietary regimen employed by these investigators is not an unusual or intolerable one and is a type of feeding program that could be used on large populations without meeting too much opposition.

### GLUCOSE METABOLISM IN THE RED BLOOD CELL

*The normal human red blood cell metabolized 11 per cent of the glucose used by way of the aerobic phosphogluconic pathway and 89 per cent by way of the anaerobic Embden-Meyerhof scheme.*

The erythrocyte is not a typical cell, since in the mature form those of mammals lack a nucleus. However, because it is easy to obtain in an uninjured form from all types of subjects and because of its keystone position in the transference of many sub-

stances, it becomes an important cell to study. Recently, J. R. Murphy (*J. Lab. Clin. Med.* 55, 286 (1960)), in the second of a series of studies on erythrocyte metabolism, reported investigations concerning the utilization of glucose.



The red blood cell can metabolize glucose to carbon dioxide and lactic acid by two different pathways. One general scheme is the anaerobic Embden-Meyerhof route of glycolysis. In this pathway, glucose is eventually metabolized to lactic acid and there is a transfer of the energy of the glucose molecules to the high energy phosphate bonds of adenosinetriphosphate. No carbon dioxide is produced in this pathway. A second route of glucose metabolism involves the aerobic phosphogluconic pathway (glucose monophosphate shunt). In this pathway, carbon dioxide is formed from the first carbon of glucose (position 1) and the energy is made available in the form of reduced triphosphopyridine nucleotide. Since the tricarboxylic acid cycle does not function or is not present in the mature erythrocytes, the only known mechanism for the production of carbon dioxide from glucose in the red cell is by way of the phosphogluconic pathway.

Murphy studied blood obtained from healthy human subjects who had not donated more than 100 ml. of blood in the previous six months. This care in choosing donors was necessary in order to have a normal age distribution of the red cell in each sample. The blood was defibrinated using glass beads and then centrifuged twice. The buffy coat was removed after centrifugation, with only the loss of a minimum number of red cells. The cells were suspended in enough autologous serum to produce a hematocrit of  $50 \pm 3$  per cent. Under these conditions there were less than 50 white cells per cubic millimeter.

The amount of glucose utilized was considered to be the difference between that present in the blood before the incubation and the amount remaining after the study was terminated. The relative and absolute amount of glucose metabolized by the phosphogluconic pathway was calculated as the difference between the total amount of glucose metabolized and the amount of  $C^{14}O_2$  recovered when the erythrocytes were incu-

bated with glucose uniformly tagged with radioactive carbon, *i.e.*, with no predominance of the tag in any one of the six carbon atoms. This substance could be used as an index since the phosphogluconic pathway is the only route for the production of  $C^{14}O_2$  from tagged glucose by the red cell.

It was found by this investigator that at pH 7.5 an average of  $11.1 \pm 0.6$  per cent of the glucose metabolized in the normal red cell occurred by way of the phosphogluconic pathway. On studying the effect of pH on the rate of utilization of glucose, it was found that over a pH range of 6.8 to 7.8 the rate of utilization of glucose by the phosphogluconic pathway did not change appreciably. However, the total utilization of glucose fell as the pH decreased. Thus the total amount of glucose utilized was 45 per cent greater at pH 7.8 than at 7.5, and when the pH was 7.1 the rate of glucose utilization fell to about one half of that occurring at pH 7.5. Since the total utilization of glucose decreased as the pH decreased, with little change in the rate of that being metabolized by the phosphogluconic pathway, it appears that under more acidic conditions a larger share of the glucose is metabolized by the phosphogluconic pathway.

On the average, 11 per cent of the total amount of glucose metabolized in the red blood cell at pH 7.5 is by way of the aerobic phosphogluconic pathway, resulting in 25 per cent of the available energy of glucose being metabolized in the form of triphosphopyridine nucleotide. On the other hand, 89 per cent of the glucose used by the red blood cell is metabolized by way of the aerobic Embden-Meyerhof scheme, resulting in 75 per cent of the energy from glucose in the form of adenosinetriphosphate.

The tracing of the different metabolic pathways of glucose in the erythrocyte is an important contribution to the physiology of this unique and important cell. Also it opens possible avenues of investigation as to the mechanism of *in vivo* hemolysis which occurs with certain drug sensitivity reactions.

## NUTRITIONAL SIGNIFICANCE OF THE RUMEN

*Rumen fermentation causes metabolism of cellulose to acetic and butyric acids, hydrogenation of unsaturated fats, and combines degradation of protein with synthesis of essential amino acids.*

The papers have now been published that were read at a symposium of the Nutrition Society in England on "Rumen Function". This has been a major field of fundamental research in British Agricultural Research Institutes for many years.

C. C. Balch (*Proc. Nutr. Soc.* 18, 97 (1959)) gave an introductory description of the physical structure of the ruminants' four stomachs, and of the observations made by cinematographic x-ray techniques on the movements of food ingesta during rumination. At the end of a meal the reticulo-rumen of a Shorthorn cow may contain as much as 200 pounds of food, and it rarely contains less than one half of this weight. Food particles, especially the larger ones may remain in the organ for several days and will only pass on to the omasum when they have become finely divided.

B. H. Howard (*Proc. Nutr. Soc.* 18, 103 (1959)) described studies made of the metabolism of food carbohydrates carried out with washed-cell suspensions of rumen bacteria. Cellulose, the most important of the polysaccharides undergoing breakdown in the rumen, is attacked mostly by bacteria with extremely strict anaerobic requirements. The main end products of their fermentation are acetic, lactic and succinic acids; but under the reducing conditions of the rumen these are further metabolized, largely to propionic and acetic acids. Fatty acids, in various proportions, seem also to be the end-products of xylan fermentation. Starch, which may be present in large amounts in rations fed to dairy cows, is also rapidly attacked by a variety of organisms present in the rumen, yielding a mixture of short-chain organic acids.

In addition, other organisms appear to synthesize and store as a reserve carbohydrate starch-like polysaccharides that give

an iodine reaction. These are not, however, entirely built up from glucose units, and one example has been shown to consist mainly of galactose, rhamnose and uronic acid units.

Discussing the problem of protein utilization, M. J. Head (*Proc. Nutr. Soc.* 18, 108 (1959)) points out that the amino acid composition of the food is less important to the dairy cow than to single-stomached species. However, it is now known that, while the rumen microflora can and do synthesize essential amino acids for condensation into microbial protein, the composition of the rumen contents passing on for digestion and absorption in the true stomach and intestines can still vary with the type of protein originally fed.

One important factor influencing the fate of protein in the rumen is its solubility. With a high level of soluble material there is considerable microbial degradation of protein with the production of ammonia which diffuses into the blood stream. Much of this is converted into urea in the liver and excreted, but some returns to the rumen via the saliva and may be re-synthesized into protein if the necessary carbohydrate energy sources are also available. One consequence of this phenomenon is that proteins can be improved for ruminant feeding by such processes as heat treatment and hardening with formaldehyde, which would have the opposite effect if the proteins were intended for non-ruminants.

In a further paper, G. A. Garton (*Proc. Nutr. Soc.* 18, 112 (1959)) summarizes the changes undergone by food lipids during their period in the rumen. Of these, the most interesting is a partial hydrogenation of the unsaturated fatty acids in the glycerides present. Measurements of changes in iodine value, reported by several workers, have all indicated that more than one half of the

double bonds initially present were reduced. However, double bonds in a conjugated state appeared to be resistant to bacterial hydrogenation; this was the case for both fatty acids and carotene. There is no evidence for synthesis of the essential fatty acids in the rumen.

Dietary fat seems of little importance in the secretion of milk fat by dairy cows, but milk deficient in fat does result from the feeding of a diet low in roughage, or with roughage in a finely ground form (J. A. F. Rook, *Proc. Nutr. Soc.* **18**, 117 (1959)). This is so even when a high level of energy continues to be fed in the form of easily digested starch. It is thought that butterfat synthesis normally occurs through acetic and butyric acids that become available from the fermentation of cellulose and other structural polysaccharides in the rumen. One would expect this fermentation to be reduced if either less roughage were fed, or if it were so finely divided that it stayed in the rumen for only a short period. Some, but not all, experiments have shown an improvement in butterfat levels after the injection of acetate or butyrate under these conditions.

Starch is, of course, also fermented in the rumen to short-chain acids, but propionic acid predominates and this acid does not appear to be used in fatty acid synthesis. On the contrary, it has a depressive effect.

Finally, A. T. Phillipson (*Proc. Nutr. Soc.* **18**, 131 (1959)) considers the implications of rumen function for the remainder of the intestinal tract and for the animal as a whole. The special system evolved for the digestion of rough food in ruminant species demands a

considerable turnover of the animal's resources. In a sheep, the daily secretion of saliva is of the order of ten liters, and of abomasal juice a further five liters. As the saliva is isotonic with plasma in its sodium content, and the quantity of sodium in most of the food eaten is small, a highly efficient system for the re-absorption of intestinal sodium is essential.

From various estimates, it appears that over 60 per cent of the dry matter in a ruminant's food is absorbed from the digestive tract while in the rumen and reticulum, *i.e.*, before the food has been in contact with the ordinary gastric and intestinal digestive enzymes. Further, estimates of the quantities of the short-chain fatty acids entering the blood stream of sheep and cattle indicate that these are, in each case, sufficient to satisfy at least the maintenance requirements for energy. The short-chain fatty acids are, of course, entirely the end-products of microbial metabolism.

Thus, concerning the nitrogenous components of the food, it is difficult to strike a balance between the losses caused by degradation of protein nitrogen to ammonia and the gains caused by the microbial synthesis of amino acids essential to the host animal.

So far, work in this field has largely been of a fundamental and exploratory nature. It can already be seen, however, that understanding of these phenomena will enable a more rational choice of feed stuffs, the processing of foods especially for ruminants, as well as feeding regimes adjusted according to the desired level of butterfat production.

### ALTERATION OF YOLK FATTY ACIDS

*Conditions have been defined by which the polyunsaturated fatty acid content of egg yolks can be varied within considerable ranges.*

According to earlier results of R. Reiser (*J. Nutrition*, **40**, 429 (1950)), the proportions of the various lipid fractions of egg yolk appear to be unaltered by the fat content of

the diet. Reiser showed that the total lipid, phospholipid and cholesterol content was the same in hens fed either a fat-free diet or one containing 4 per cent of cottonseed oil.

However, from theoretical considerations, the fatty acid composition of egg yolk may well vary as a function of the diet. It is, therefore, not surprising that somewhat variable results have been obtained by different workers who have analyzed the fatty acid composition of egg yolk. The analyses of R. W. Riemenschneider *et al.* (*J. Biol. Chem.* **126**, 255 (1938)) differ from those reported by F. B. Shorland (*New Zealand J. Sci. & Technology* **33B**, 224 (1951)). Of course, analytical methods and genetic differences may account, in part, for such differences, but the egg yolks analyzed by Shorland were from hens of unknown dietary history, whereas those analyzed by Riemenschneider *et al.* were from hens that had been fed 2 per cent of cod liver oil and 7 per cent of fish meal (which may account for the relatively high level of C<sub>22</sub> unsaturated fatty acids found).

E. M. Cruickshank (*Biochem. J.* **28**, 965 (1934)) and Reiser (*J. Nutrition* **44**, 159 (1951)) present evidence that the unsaturated fatty acid portion of the egg yolk is most susceptible to dietary manipulation. Cruickshank reported variations in fatty acids of egg yolks from hens fed high levels (28 per cent) of various fats. On feeding hens palm kernel oil (a highly saturated fat) she obtained only 16.1 per cent of the total fatty acids as linoleic acid, but on feeding a highly unsaturated fat (hemp oil) the linoleic content of the yolk was 41.7 per cent. Reiser showed that either a fat-free diet or the presence in the diet of bayberry tallow would result in reduction of the dienoic acid content of the yolk to very low levels. Even at low dietary fat levels (2 per cent) the polyunsaturated fatty acid fraction could be increased by feeding tung oil or codliver oil.

A study by A. S. Feigenbaum and H. Fisher (*Arch. Biochem. Biophys.* **79**, 302 (1959)) demonstrated that when various oils were fed to hens at 10 per cent of the diet the composition of the yolk fat was influenced only by the polyunsaturated fatty acids, whereas the composition of the body

fat was influenced by either saturated or unsaturated fatty acids.

With the advent of gas-liquid chromatography, an improved analytical technique can be brought to bear on this problem. P. D. Wheeler, D. W. Peterson and G. D. Michaels (*J. Nutrition* **69**, 253 (1959)) have fed hens diets containing 5, 10, 15, 22.2 or 30 per cent of safflower oil; 22.2 or 30 per cent of linseed oil; and 10 per cent of cottonseed, corn or soybean oil. The total fatty acids of the yolk fat were determined by alkaline isomerization and by gas-liquid chromatography and their distribution compared with that of eggs produced by hens on a stock diet or on a low-fat diet.

The results of this study corroborated those of Reiser (*J. Nutrition*, **40**, 429 (1950)) in that the total lipid and free and esterified cholesterol content of the eggs from hens fed up to 30 per cent of oil did not differ from the values obtained with eggs from hens on the stock or control diet. However, in contrast to the findings of Reiser, the phospholipids were found to be considerably higher on the high-fat diets. Eggs produced by hens on a 20 per cent safflower oil diet averaged 1.8 g. heavier than those from hens on a stock diet. The flavor of the "safflower eggs" did not differ from that of control eggs, but eggs from hens on high linseed oil diets were reported by a taste panel to have off-flavors.

It was found that the linoleic acid content of eggs from hens fed safflower oil or cottonseed oil was approximately proportional to the level of this acid in the diet. However, in eggs from hens fed diets containing linolenic acid (including corn oil, soybean oil and linseed oil) linoleic acid was not deposited as efficiently as in those from hens fed safflower oil or cottonseed oil. The authors suggest an antagonistic or inhibitory effect of linolenic acid upon the incorporation of linoleic acid into yolk fat.

Analyses of eggs from hens on the linseed oil diet revealed that the increases in yolk levels of unsaturated fatty acids were ac-

accompanied by decreases in the monoenoic acids and palmitic acid but that the proportion of stearic acid remained the same. On all other diets, the principal changes accompanying increases in linoleic acid were decreases in oleic and palmitoleic acids. Only at about 40 per cent of linoleic acid was a slight decrease in palmitic acid observed and no change occurred in the proportion of stearic acid.

The levels of tetraenoic, pentaenoic and hexaenoic acids were low and variable in all diets. The authors could ascribe none of the changes to the diets used in these experiments.

It appears from the foregoing that conditions can now be defined which make it possible to vary the linoleic acid content of eggs. In the future, we can expect similar applications to a variety of grown foodstuffs.

### INCAP ALL-VEGETABLE PROTEIN MIXTURES FOR HUMAN FEEDING

*Biologic testing with rats and chicks has demonstrated the efficacy and safety of high-protein indigenous vegetable mixtures prior to trials with infants and children.*

The manufacture of vegetable protein mixtures from low-cost ingredients available or potentially available in a given geographic region provides a practical answer to the problems of protein malnutrition, particularly in children, in many areas of the world. In planning such mixtures, at least eight factors must be considered (M. Béhar *et al.*, *Ann. N. Y. Acad. Sci.* **69**, 954 (1958)): (1) the amino acid content of the individual ingredients and the final product; (2) the possible presence of toxic or interfering factors; (3) the need for obtaining exact specifications of each of the components; (4) the necessity of avoiding processes that damage the quality of the protein; (5) the desirability of using products of local origin; (6) the fact that the final product must be inexpensive and easily preserved; (7) the requirement that it be easily prepared in the home as an infant food by mothers of low-income families; and (8) the demand that it must not run counter to existing dietary habits and prejudices.

In addition to these requisites, the product should not be recommended for commercial production and mass consumption until four types of biologic trials have been carried out: (1) testing the product in at least two animal species; (2) demonstration of the biologic value of its protein by animal growth studies; (3) testing its acceptability and effective-

ness in careful studies with children; and (4) field trials in selected communities or small population groups.

These principles and procedures have guided the workers at the Instituto de Nutricion de Centro America y Panama (INCAP) in developing a vegetable mixture, now entering commercial production, for the prevention of protein malnutrition in infants and young children in Central America. The history of the development of this all-vegetable protein mixture for human feeding has begun to be detailed in a new series of publications from INCAP (R. L. Squibb, M. K. Wyld, N. S. Scrimshaw and R. Bressani, *J. Nutrition* **69**, 348 (1959); Bressani, A. Aguirre and Scrimshaw, *Ibid.* **69**, 351 (1959)).

The first paper describes in part the biological trials in rats and baby chicks of various food ingredients and combinations which preceded trials with children. Four independent trials were carried out with weanling white rats of the IAN (Instituto Agropecuario Nacional, Guatemala) Wistar strain colony. Three independent trials were carried out with three-day-old, straight-run baby New Hampshire chicks. The diets tested all contained approximately 25 per cent of crude protein (the protein level desired in a product for infant and child feeding) and were composed of crude sesame oil meal or refined

sesame flour, lime-treated corn or ground raw corn, cottonseed flour or dried skim milk, kikuyu leaf meal (as a source of vitamin A), *Torula* yeast or a B-complex vitamin mixture, vitamin D or cod liver oil, and (in certain trials) L-lysine, DL-valine, arginine, gelatin and corn cob meal.

During trial one of the rat-feeding experiments, a comparison was made between crude sesame oil meal and refined sesame flour for a nine-week period with and without the addition of skim milk. Data analysis revealed no significant differences in the weights of the rats fed the two sesame products, with or without added skim milk. Refined sesame flour without skim milk, however, resulted in an efficiency of feed utilization (gram feed per gram gain) superior to that with crude sesame oil meal, but when skim milk was added to both types of sesame there was no difference in efficiency.

During rat trial two, cottonseed flour (9 per cent) and two levels of dried skim milk (9 and 14.3 per cent) were tested as supplements to the sesame flour for a period of eight weeks. The supplements tested were substituted for a part of the protein of the sesame flour. Rats receiving the higher skim milk supplement gained slightly more and showed a better feed efficiency, but the differences in growth were not statistically significant.

During rat trial three, cottonseed flour and dried skim milk, with and without L-lysine, were tested as supplements to sesame flour in the all-vegetable mixture for a period of eight weeks. As before, the supplements were substituted for a part of the protein of the sesame flour. The data showed no significant differences in the growth of the rats among the experimental and control groups. However, in each instance, the addition of L-lysine improved the efficiency of feed utilization. The authors attributed the lack of significant growth stimulation by lysine to the high protein content (25 per cent) of the diets and to the fact that the experiment was continued beyond the usual four-week period of rapid growth response.

During rat trial four, the effects were tested of supplements of L-lysine and DL-valine (the amino acids most deficient in the all-vegetable mixture when compared to milk) in diets containing 15 per cent protein derived mainly from sesame and cottonseed flour. A depression of growth rate and feed efficiency, not significant at the 5 per cent level, was noted on addition of DL-valine. A significant increase in growth rate and feed efficiency was observed on addition of L-lysine.

During trial one of the chick-feeding experiments, the effect of lime treatment of corn on chick growth and feed efficiency was observed by comparing lime-treated and raw corn (with and without supplementation by B-complex vitamins) as components of the all-vegetable mixture containing sesame and cottonseed flours. Raw corn produced significantly greater growth and efficiency of feed utilization whether or not B-complex vitamins were added. The greater growth occasioned by the addition of B-complex vitamins was not statistically significant.

During chick trial two, the effects of adding gelatin, arginine and lysine to a sesame flour, cottonseed flour, lime-treated or raw corn combination were measured. Gelatin was tested because it is a good source of arginine for the chick and because of the possibility that the inferior growth obtained with lime-treated corn might be due to the loss of 18 per cent of arginine occurring as a result of lime treatment (Bressani and Scrimshaw, *J. Agr. Food Chem.* 6, 774 (1958)). Addition of gelatin decreased growth and feed efficiency, but the addition of arginine increased both, none of the differences, however, reaching the 5 per cent level of significance. Addition of L-lysine, however, singly or in combination with the other supplements tested, significantly increased growth and feed efficiency of the lime-treated corn diets.

Since chick trials one and two had indicated that the sesame flour, cottonseed flour, lime-treated corn combination was lysine de-



ficient for chicks and that the use of lime-treated corn resulted in lower growth rates in chicks, an attempt was made during chick trial three to improve digestion and absorption through the purely mechanical effect of giving three levels of corn cob meal in place of part of the lime-treated corn to three of eight groups of chicks. In another three of the eight groups tested, the supplemental value of L-lysine was tested at 0.2, 0.3 and 0.4 per cent of the diet. Lime-treated and raw corn were compared directly in the remaining two groups.

Lime-treated corn again produced lower growth. Addition of corn cob meal to lime-treated corn diets improved chick growth to values comparable to those obtained with raw corn. L-lysine addition produced greater growth and feed efficiency at all levels, but had its greatest effect on growth at the 0.4 per cent level. The authors interpreted these results to indicate that the physical characteristics of the raw corn diets, rather than any direct nutritional effects, were responsible for their superiority for chicks over the lime-treated corn diets.

These experiments and other previously collected laboratory data led to the adoption of a final formula for human trials. This formula, designated INCAP Vegetable Mixture 8, contained in per cent: dry corn masa, 50 (masa is obtained by cooking corn in a lime solution and subsequently drying and grinding it); sesame flour, 35; cottonseed flour, 9; kikuyu leaf meal, 3; and *Torula* yeast, 3. The mixture contained adequate vitamins and minerals (Béhar *et al.*, *Ann. N. Y. Acad. Sci.* **69**, 954 (1958)). Comparison of the essential amino acid pattern of this formula with the pattern of cow's milk (M. L. Orr and B. K. Watt, *Home Economics Research Report No. 4*, U. S. D. A., Washington (1957)) and the FAO Reference Protein (*Food and Agriculture Organization Nutritional Studies No. 16*, p. 24 (1955)) suggests that the INCAP mixture is limiting in lysine, that tryptophan is the second most limiting amino acid and that methionine (methionine plus cystine) is

the third. The protein score of the mixture is about 67 per cent, indicating that the mixture is within the lower range of protein of animal origin.

The biologic trials described satisfied the authors as to the lack of toxicity of any of the ingredients or combinations of ingredients in the mixture and encouraged the probability that the mixture would prove adequate for the nutritional needs of growing infants and children. Subsequent experience in infants and young children, to be detailed later in this series confirms the adequacy of the rat and chick testing methods in evaluating vegetable mixtures.

The second paper in this series (Bressani, Aguirre and Scrimshaw, *J. Nutrition* **69**, 351 (1959)) describes experiences of INCAP workers in feeding chicks Vegetable Mixture 8 in which the lime-treated corn of the original mixture was replaced by raw yellow corn, grain sorghum (one species of a genus of tropical cereal grasses), rice or buckwheat. Each cereal grain tested was substituted completely for 50 per cent of the corn masa flour of the basal diet. The complete diets were diluted to 75 or 80 per cent of their original value to give protein concentrations of approximately 21 or 22 per cent.

Buckwheat resulted in better growth, feed efficiency and protein efficiency (gram of weight gained per gram of protein consumed) than any of the other cereal grains substituted for part of the corn. Raw yellow corn and rice produced slightly greater growth than sorghum and the basal INCAP Vegetable Mixture 8. Feed efficiency values for the basal diet, however, were somewhat better than those obtained when yellow corn and rice were utilized. Feed efficiency and protein efficiency values of the rations containing sorghum were the lowest. Protein efficiency values of the basal diet, yellow corn and rice were essentially equal. Lysine supplementation at the 0.4 per cent level improved the growth, feed efficiency and protein efficiency of all the experimental rations, being most effective during rice substitution.

The results of these experiments suggest that any of the four cereals tested could be substituted for all or part of the corn masa flour in INCAP Vegetable Mixture 8 if economic and agricultural factors make this desirable. They also point up interesting problems for future investigation, particularly whether the differences observed among the variously substituted mixtures are due to differences in amino acid availability, a less favorable amino acid pattern or lower digestibility.

Preliminary reports have been published (Scrimshaw *et al.*, *Amino Acid Malnutrition*, XIII, p. 28. *Annual Protein Conference*, Rut-

gers University Press (1957); Béhar *et al.*, *Ann. N. Y. Acad. Sci.* **69**, 954 (1958)) which indicate good results from the feeding of INCAP Vegetable Mixture 8 to young children. More extensive clinical experience, to be detailed in the next paper in this series, bears out the impressions expressed in the preliminary reports. The development of "Incaparina" should serve as a model for similar work elsewhere as well as a warning that long years of carefully planned and deliberately executed research are requisite to obtaining an effective and thoroughly safe product for human consumption.

### PLASMA AND BONE MAGNESIUM

*A relationship between bone-ash magnesium and plasma magnesium has been demonstrated. However, the bone plays little or no part in the control of plasma magnesium in magnesium excess, but serves as a reservoir in deficiency.*

It was shown by E. R. Orent, H. D. Kruse and E. V. McCollum (*J. Biol. Chem.* **106**, 573 (1934)) that bone magnesium is depleted in rats kept on magnesium-deficient diets. The same was subsequently shown to be true for calves (C. E. Knoop, W. E. Krauss and C. C. Hayden, *J. Dairy Sci.* **22**, 283 (1939); W. H. Parr, *Vet. Rec.* **69**, 71 (1957)), but in neither of these animals are the soft tissues appreciably depleted even under the most severe conditions of magnesium deficiency.

It thus appears that bone magnesium represents a store of this element that can be called upon under conditions of deficiency to supply the needs of the soft tissues. J. Duckworth and W. Godden (*Biochem. J.* **35**, 816 (1941)) have shown, by killing rats at different times after introducing a magnesium-deficient diet, that bone magnesium can be mobilized rapidly. K. L. Blaxter (*Ciba Foundation Symposium on Bone Structure and Metabolism*, p. 117. Churchill, London (1956)) has related bone magnesium to plasma magnesium in calves slaughtered with differing degrees of hypo-

magnesemia developed as a result of protracted feeding of milk.

During a study by R. H. Smith (*Biochem. J.* **71**, 609 (1959)) on the development of hypomagnesemia in milk-fed calves, the opportunity was presented for a very detailed analysis of the relationship between such hypomagnesemia and changes in bone composition. Bones examined were the caudal vertebrae (which could be taken from the living animal), rib shaft, femur head and shaft and the first phalanges. At least ten successive samples could be obtained from one calf if sections containing only one vertebra were removed at a time.

The bone compositions in several calves fed normally and in two groups of calves developing hypomagnesemia were examined. The first group of calves developing hypomagnesemia consisted of animals fed almost exclusively on cow's milk supplemented with vitamin D, iron, copper and manganese. Caudal vertebrae were removed at approximately monthly intervals. The second group consisted of calves which, at about two to four weeks of age, were transferred

from a magnesium adequate diet to one of low-magnesium synthetic milk. Caudal vertebrae were removed before and after the change in diet. Preliminary experiments showed that compositions of most of the vertebrae in an individual animal were similar, so that one vertebra could be used to assess the composition of the remainder.

Although the ash contents of the different types of bones in the animal were somewhat variable, the only marked consistent difference was in the femur, which contained 20 to 30 per cent more ash than did the caudal vertebrae in both the normal and magnesium-depleted calves. The calcium in the ash was closely similar for the different bones in all the calves, as was the magnesium in the ash for the normal calves. In the magnesium-depleted calves there were marked differences among the ashes of the various bones. Of those studied, the femur shafts were depleted to the least extent and the vertebrae and first phalanges to the greatest extent. The author points out that the caudal vertebrae, therefore, offer a sensitive guide to the magnesium status of the skeleton, but that changes observed in these vertebrae during magnesium depletion do not exactly reflect the changes occurring in the skeleton as a whole.

There appeared to be a broad direct relationship between the rate of onset of hypomagnesemia in the different calves and the rate of depletion of their bone magnesium. Thus, drops in concentrations of magnesium in the plasma to 1.6 and 0.7 mg. per 100 ml. were associated with bone-ash magnesium figures of 0.60 to 0.67 and 0.40 to 0.48 per cent, respectively, whatever the rate at which the concentration of plasma magnesium fell. The plasma magnesium decreased only very slowly after reaching a level of about 0.7 mg. per 100 ml., and further depletion of bone magnesium occurred with relatively small decreases in the plasma concentration. When the last bone samples were taken (some animals died, presumably from magnesium

deficiency) the mean plasma magnesium level was 0.47 mg. per 100 ml. and the mean magnesium in the bone ash was 0.27 per cent (about 38 per cent of normal).

Following an abrupt change to a low-magnesium diet, the depletion of bone magnesium appeared to occur at an approximately constant rate (presumably at a rate necessary to provide adequate magnesium for the growing soft tissue and for endogenous fecal excretion). Urine excretion of magnesium fell to nearly zero as soon as the calves were transferred to a magnesium-deficient diet. Plasma magnesium fell, rapidly at first and then more slowly. The growth rates of the calves and their consequent needs for magnesium were depressed.

When the bone-ash magnesium values of two of the calves fed on low-magnesium synthetic milk had fallen, respectively, to 84 and 68 per cent of the normal value, they were given daily injections of one gram of magnesium (sulfate). After five days of injections, the plasma magnesium of both calves ranged between 3.7 and 2.2 mg. per 100 ml. (about 160 and 95 per cent of normal). Following nine days of injections in one case and 16 days in the other, the bone-ash magnesium for both calves was found to be normal. Bone samples (vertebrae) taken periodically from each of the calves did not show any marked changes in ash content nor in the percentage of calcium in the ash concomitant with magnesium depletion.

The author points out that the above results suggest that magnesium behaves similarly to calcium in its lack of uniform depletion of different parts of the skeleton during deficiency. Calcium has also been shown to be removed more readily from cancellus bone than from compact bone in calcium-deficient sheep (D. Benzie *et al.*, *J. Agr. Sci.* **46**, 425 (1955)). The relationship between bone and plasma magnesium in calves slaughtered with varying degrees of hypomagnesemia is similar in general to that reported by K. L. Blaxter (*loc. cit.*).

Such small differences that exist may be partially accounted for by the fact that Blaxter examined bone from the metatarsal shaft whereas Smith used caudal vertebrae.

The results obtained suggest further that an equilibrium exists between bone and plasma magnesium and that normal bone, from a physiological standpoint, is virtually saturated with magnesium. This was especially indicated by the fact that daily injection of magnesium, so that the plasma magnesium did not fall appreciably below normal (and for a part of the time was considerably above normal), did not lead to any appreciable increase in bone magnesium above normal amounts.

Thus the bone plays little or no part in regulating concentration of plasma magnesium. Instead this control seems to be largely a function of changes in urine excretion. The excretion is high when the diet

contains more than adequate magnesium (Smith, *Biochem. J.* **67**, 472 (1957); **70**, 201 (1958)) and increases enormously when enough magnesium is injected so that plasma magnesium rises above normal (Smith, *Ibid.* **71**, 306 (1959)). However, it decreases, eventually nearly to zero, when the magnesium available from the diet is decreased. Only when the available magnesium is so low as to be inadequate even with almost no loss in the urine, is bone magnesium apparently liberated.

M. J. Dallemagne and C. Fabry (*Ciba Foundation Symposium on Bone Structure and Metabolism*, p. 14. Churchill, London (1956)) have suggested that about two-thirds of bone magnesium is adsorbed on the surface of the bone-salt crystals, with the remaining one-third replacing calcium in the phosphate molecule. The author suggests that the adsorbed fraction may be, in fact, that portion which is available in magnesium depletion.

### ABSORPTION OF CYSTINE AND CYSTEINE

*The absorption of isomers of cystine and cysteine from the small intestine of the rat has been studied with a lumen-perfusion (in situ) technique and with an everted-sac technique.*

Many worthwhile studies have been made of the efficiency of specific proteins in promoting growth, maintaining the nitrogen balance and supporting good health. However, for a basic understanding of the mechanism involved, it is necessary to know where and at what rates the various amino acids are absorbed from the intestine.

Although numerous investigations have been made in the past few years of the absorption of amino acids from the small intestine, cystine and cysteine have not been included in the compounds studied. This is due in part to the insolubility of cystine and the rapid oxidation of cysteine in oxygenated solutions. Recently, M. W. Neil (*Biochem. J.* **71**, 118 (1959)) has employed a lumen-perfusion technique *in situ* which

permits observation of absorption of amino acids in a defined segment under purely physiological conditions. The everted-sac technique was also used by Neil in some studies of cystine absorption.

Male albino rats weighing 200 to 300 g. were used, and dissection, intestinal cannulation and perfusion were carried out by conventional techniques. The perfusion pressure measured at the proximal end of a perfused segment was kept at 9 to 11 cm. water throughout each experiment with a total volume of the fluid (bicarbonate saline) amounting to about 55 ml. One ml. samples were removed periodically for analysis. Added cystine plus cysteine were determined polarographically with Tinsley polarographic equipment and then cystine

alone was measured after allowing the cysteine to react with iodoacetate.

In perfused segments of duodenum, jejunum and ileum, the potassium content of the whole tissue remained practically normal. The water content of the segments was only about 3 per cent higher than that of control samples. Apart from this slight edema, the mucosal epithelium was practically normal in sections from perfused segments. The well established inhibition of active absorption of glucose by phlorizin in such preparations was used as a functional test. Inhibition was unequivocally demonstrated.

Absorption of cystine and cysteine was studied by following the decrease of the amino acid in the perfusion fluid. The big advantage (as far as cysteine is concerned) of the preparation *in situ* is that it is possible to keep the perfusion fluid oxygen-free as the gut itself is oxygenated by the flow of blood.

In the jejunum the rate of absorption of L-cystine was found to be about 70 per cent of that of L-cysteine. Substitution of nitrogen for oxygen in the cystine absorption experiments had no effect on the rate of absorption of this amino acid. The rates of absorption of cystine clearly decreased in the order: duodenum, jejunum and ileum. The absorption of L-cysteine was also slower from the jejunum than from the duodenum.

The D-isomers of both cystine and cysteine were absorbed from rat duodenum at much lower rates than their L-isomers. These results provided evidence that an active process was involved in the absorption mechanism for the L-isomers in each case.

Since certain preliminary experiments indicated that the physiological state of the rat small intestine was reasonably well preserved with the everted-sac technique, the author decided to use this preparation for further studies on the nature of the transport of cystine across the rat intestinal wall.

Everted sacs of rat small intestine were

prepared and incubated in bicarbonate saline, containing cysteine. The initial cystine serosal to mucosal concentration ratio was 1:0. After one hour the ratio was again determined. It was found that L-cystine was transported from the mucosal side to the serosal side of the sacs against this concentration gradient. No such transport of D-cystine occurred.

Since dinitrophenol inhibits phosphorylating processes that might be involved in active transport, the effect of this substance was ascertained with both the lumen-perfusion techniques and the everted-sac techniques. In the first instance, the presence of 0.3 millimolar 2,4-dinitrophenol had no influence on the absorption of D-glucose, L-cystine and L-methionine and did not alter the potassium and water contents of the perfused segments. However, in the everted-sac technique, such a large loss of potassium and increase in water occurred that the test for inhibition of cystine absorption was ruled invalid.

The author emphasizes the importance of comparing the results of absorption studies on the basis of the particular technique used (this should also be kept in mind when extrapolating for nutritional applications). For example, in the present case, the absorption routes of the metabolites were different in the two preparations. In the technique *in situ* the route approximated the normal, but in the everted-sac technique the amino acids had to traverse the entire intestinal wall. However, the conditions were more reproducible in the technique *in vitro* than in the technique *in situ* because the latter was subject to such factors as variation in the blood flow perfusing the gut capillaries, amounts of anesthetic required, degree of shock and degree of distention of the segment during the perfusion.

Unfortunately, from the standpoint of physiological nutritionists, the author presents no evidence citing the relative absorption of glucose or L-methionine as compared to L-cystine and L-cysteine.

AGRICULTURE

## LEAF PROTEIN AS HUMAN FOOD

*A simple method of extracting protein from plants has been devised and the resulting protein product, while deeply colored with chlorophyll, is odorless, tasteless and suitable for protein supplementation in man.*

In spite of the millions of hungry people in the world today, world population is increasing by about 40 to 50 million persons a year. When modern medicine and sanitation become more effective in the undeveloped regions this rate will undoubtedly increase, and with this there will be an increasing demand for all nutrients. Although the employment of improved methods of agricultural technology, increased irrigation and drainage, selection of crops to produce a greater yield per acre, and the eradication of plant disease and pests will lead to greater production of foodstuffs, there is doubt whether such methods can meet the increased demand for food. Protein will probably be in shortest supply since the foodstuffs that are easiest to produce, yield large amounts of calories but supply inadequate amounts of protein. Moreover, when foodstuffs are limited in supply the use of animals for converting plant protein into animal protein is too inefficient. Hence, in large areas of the world animal protein is used as a condiment rather than a source of utilizable nitrogen.

Recently, N. W. Pirie (*Lancet* II, 961 (1959)) has discussed the possibility of converting leaf proteins into foodstuffs suitable for human consumption. It is logical to use such products since all of the proteins derived from animals have been produced by the animal organism directly or indirectly from plants. Thus a device has been made which simply and efficiently extracts proteins from plant products.

Fresh green leaves are first pulped and the juice expressed from the solid material. After straining the juice, the protein is coagulated by heating to 80°C and filtered off. The protein is then resuspended in water and filtered again. If the original leaf were strongly flavored this process can be

repeated. The protein cake resulting from this procedure, while dark green in color due to the chlorophyll present, has little or no taste or smell and has the consistency of cheese or yeast. It contains 60 per cent water. At room temperature it will keep a week or so but slowly molds. Drying in air or in an oven yields a hard granular product, but it is possible to avoid this undesired consistency by either freeze-drying or drying it in the presence of flour or other materials. The plant proteins will keep indefinitely in the frozen state and can also be canned for storage.

One of the advantages of these protein products is that they are a mixture of a number of different plant proteins and, therefore, probably contain a better spectrum of amino acids. Thus the amino acid deficiency which may be present in any single plant protein tends to be modified by the presence of other proteins. Pirie believes that these proteins will be a valuable food even as the main protein of the diet, although not as good nutritionally as the standard animal proteins, casein or egg albumin. When nutritional studies were carried out on pigs it was found that the leaf proteins were as good or a little better than fish meal. Pirie also indicates that comparable findings (not yet published) were noted for chickens and rats.

Now that it has been shown that proteins fit for human consumption can be readily formed from many types of plant products, the question arises as what could best be used as raw material in areas where the need for proteins is most acute. The author suggests that there are, in general, three main types of leaves which could be used effectively. The first are wild leaves, but since these normally grow in areas unsuitable for large scale cultivation, collection



would be difficult. However, water plants such as papyrus and the water hyacinth (which in many cases are clogging waterways) would be easy to collect and would supply a ready source of protein. In addition, this process would help clear rivers and bays. The second type are the waste leaves, such as sugar beet tops in the temperate zones, while in the warmer regions the leaves of such crops as sweet potatoes, sugar cane, banana and cassava might be good sources of leaf protein. Technical difficulties are foreseen with some of these products, but these may be solved as they arise.

Finally, it would be possible to plant special crops to be harvested mainly for leaf proteins. There are many plants which grow luxuriously but, because they do not produce large seeds or tubulars or the leaves are unpalatable to livestock, cannot be used at present for food. Also, by raising special crops to obtain leaf proteins it might be possible in many cases to harvest the plants

while young and less vulnerable to some pests and diseases which attack plants in the more mature state.

Obviously some of the difficulties which may arise in using these plant proteins on a large scale are food habits and preferences, particularly since the material is intensely green in color. This alone may prevent its widespread use if there is insufficient education. Also if 20 g. or more of such a protein is injected, part of the chlorophyll escapes and appears in the feces. The colored stool does not indicate that the protein is not being digested but rather that the chlorophyll is not being destroyed.

This simple device for extracting protein from plant leaves opens new areas in providing nutrition for a large population. Also the possibility exists that increased utilization of other parts of plants, presently harvested for only seeds or fruit, might be used to increase the protein supply of an area.

## PHOSPHATIDE-INDUCED HYPERCHOLESTEREMIA

*The results of experiments on laboratory animals suggest that intravenous infusions of phospholipids may cause mobilization of tissue cholesterol.*

Infusions of various types of phospholipids have been shown to increase the concentration of cholesterol in the plasma of rats and rabbits (M. Friedman and S. O. Byers, *Proc. Soc. Exp. Biol. Med.* **94**, 452 (1957); *Am. J. Physiol.* **186**, 13 (1956)). Hypercholesteremia develops when a solution of phosphatides from liver, brain, soybeans or yeast is infused over a six to 24-hour period (Byers and Friedman, *Am. J. Physiol.* **193**, 435 (1958)). In an attempt to elucidate the mechanism of this phosphatide-induced hypercholesteremia, Friedman and Byers (*Ibid.* **195**, 185 (1958)) studied the effects of infusions of lecithin on the cholesterol content of various organs of the rat.

Rats were given intravenously, without anesthetic, 3 ml. of a colloidal suspension

of lecithin (3 per cent in a solution containing 5 per cent of dextrose) at the beginning of the experimental period. Thereafter they were infused continuously at a rate of 0.5 ml. per hour for 12- or 24-hour periods. Cholesterol concentration was determined by the method of A. Saifer and O. F. Kammerer (*J. Biol. Chem.* **164**, 657 (1946)), in whole blood, plasma and tissues. Blood was obtained by bleeding the animals from the tail.

When a laboratory chow diet to which 3 per cent of cholesterol and 4 per cent of cottonseed oil had been added was pre-fed, the infusion of lecithin brought about a significant reduction in the concentration of cholesterol in the liver within 12 hours. However, when a sterol-free diet was fed,

the liver cholesterol concentration of rats infused for 24 hours with lecithin was higher than that of rats infused with dextrose alone or of rats not subjected to infusion. The infusion of dextrose alone resulted in some lowering of liver cholesterol concentration.

Twenty-four-hour infusion of lecithin resulted in a 75 per cent decrease in the cholesterol content of the adrenals of rats pre-fed a sterol-free diet. However, infusion of dextrose alone reduced the adrenal cholesterol concentrations from 121 mg. per g. to 54 mg. per g., a 55 per cent decrease. In rats pre-fed the cholesterol-enriched diet, a 12-hour infusion with lecithin resulted in a cholesterol content of the adrenals of 32 mg. per g. while infusion with dextrose alone gave a value of 55 mg. per g. Unfortunately no values were reported for control rats pre-fed the cholesterol-enriched diet but given no infusion. Infusion of lethicin or dextrose alone into rats fed either the sterol-free diet or the cholesterol-enriched diet caused no change in cholesterol concentration of the intestines, skin, lung and other viscera studied.

Analyses of whole blood following infusion showed an increase in cholesterol concentration of the same magnitude as that occurring in plasma, indicating that the excess plasma cholesterol did not originate in the formed elements of the blood. Also, the possibility that cholesterol or other sterols present in the phospholipid which was infused might account for the hypercholesteremia must be discounted because especially purified samples of phospholipid, essentially free of Lieberman-Burchard positive sterols, were as effective in producing hypercholesteremia as samples that were not highly purified.

In the interpretation of these results, the authors conclude that the infusion of phosphatides can reduce the cholesterol content of organs having a high concentration of cholesterol, such as the adrenals and the liver, the latter only in animals pre-fed a

cholesterol-rich diet. They believe that the infused phospholipid has a "solubilizing or chemotaxic" effect on cholesterol in various tissues including aortic atherosclerotic plaques (Friedman, Byers and R. H. Rosenman, *Proc. Soc. Exp. Biol. Med.* **95**, 586 (1957)), thus transferring tissue cholesterol to the blood. However, the amount of cholesterol removed from the various organs studied was not sufficient to account for the amount of cholesterol found in the plasma after phospholipid infusion. The authors suggest that the depletion of 10 to 21 mg. of cholesterol (the amount required to account for the observed hypercholesteremia) from the total body pool of 300 to 400 mg. could not be detected by the quantitative determination used.

They discount an effect of infused phospholipid on the metabolism or mobilization of cholesterol in the liver because the infusion of phosphatide resulted in even higher plasma cholesterol levels in hepatectomized rats than in normal animals (Byers and Friedman, *Proc. Soc. Exp. Biol. Med.* **92**, 459 (1956)). The plasma cholesterol concentrations were higher in hepatectomized rats presumably because less of the phospholipid was removed from the blood in the absence of the liver and the resulting higher plasma phospholipid concentration caused more severe hypercholesteremia.

Since prefeeding a cholesterol-rich diet enhances the hypercholesteremia of phosphatide infusion (Friedman and Byers, *Am. J. Physiol.* **192**, 546 (1958)) and since dietary cholesterol depresses cholesterol synthesis, the authors have taken this as additional evidence against the possibility that increased cholesterol synthesis is responsible for the phosphatide-induced hypercholesteremia.

The hypothesis of Friedman and Byers concerning the origin of excess plasma cholesterol in phosphatide-induced hypercholesteremia appears reasonable, but the possibility that changes in cholesterol metabolism occur secondarily to hypercholesteremia has

not been eliminated. A small increase in synthesis in several or all organs from which cholesterol is removed by the lipemic serum could restore the original tissue concentration and account for the fact that little change is observed in the over-all tissue concentrations of cholesterol when the serum level rises. However, direct studies of the effects of infusion of phospholipids on the synthesis and catabolism of cholesterol are needed to appraise this possibility.

In evaluating the conclusions, the possible effects of the stress to which the animals were subjected must also be considered, par-

ticularly in view of the reduction in liver and adrenal cholesterol demonstrated upon infusion of dextrose alone; however, infusion of dextrose alone produced no hypercholesteremia.

These observations on the effects of phospholipid infusions on serum and tissue cholesterol concentrations appear to have received little attention. They should be investigated in more detail, particularly in view of the possibility that the infusion of phospholipid may lead to the removal of cholesterol from atherosclerotic plaques.

### RIBOFLAVIN AND ADRENAL CORTICAL METABOLISM

*Rats fed a diet deficient in riboflavin developed signs compatible with ariboflavinosis, i.e., impairment of adrenal ascorbic acid depletion following stress. However, pair-fed control animals showed a similar response.*

Although riboflavin was found by P. György to be necessary for growth of rats (*Nutrition Reviews* 12, 97 (1954)), the details of its mode of action were unknown. One of its functions seemed to relate to the adrenal cortex. Animals deficient in riboflavin were found to have lost their glucocorticogenic response to anoxia (B. R. Forker and A. F. Morgan, *J. Biol. Chem.* 209, 303 (1954); 217, 659 (1955); *Nutrition Reviews* 13, 19 (1955)), but this could be restored by administration of riboflavin or of cortisone. Another report described the inability of deficient animals to show diuresis after being loaded with water (*Nutrition Reviews* 14, 120 (1956)). This defect also was corrected by cortisone or corticotrophin.

These considerations led G. G. Slater (*Endocrinology* 65, 731 (1959)) to study further the role of riboflavin in the metabolism of the adrenal cortex in animals, as well as the effect of total dietary restriction on this function. He chose rats weighing 50 to 200 g. and fed them (1) a high-carbohydrate, riboflavin-free diet, or (2) a high-protein, riboflavin-free diet. Galactoflavin, a metabolic antagonist to riboflavin, was

added to the ration of some animals in the amount of 1 mg. per gram of diet. Others were given riboflavin in their ration. Stress was applied by surgically removing the left adrenal gland and, one hour later, the right adrenal. The ascorbic acid content of each adrenal was then determined.

In the normal animal, this stress significantly reduced the content of adrenal ascorbic acid. In the present study, loss of this response was considered evidence of riboflavin deficiency. In young animals (weighing 80 g.) the response was lost after ten days of a diet high in carbohydrate and containing galactoflavin. In older animals the response became impaired after three to five weeks.

Severity of the stress seemed to be a factor, and excessively severe surgical manipulation was capable of evoking depletion of adrenal ascorbic acid in a group of animals which did not respond to the milder operative procedure.

Dietary intake also was important. When control groups of animals were allowed only enough food to maintain their weight within a range similar to that of the deficient

animals, this restriction of their total diet checked the depletion of adrenal ascorbic acid.

Intraperitoneal administration of 1.5 mg. of riboflavin to deficient animals failed to restore their response to stress applied three hours later. It was also significant that pair-fed controls (one group deficient and the other supplemented with riboflavin) displayed a similar degree of impairment of adrenal ascorbic acid depletion following stress.

Forker and Morgan (*loc. cit.*) had earlier demonstrated that depletion of adrenal ascorbic acid failed to occur in riboflavin-deficient rats subjected to stress, whereas pair-weighted but riboflavin-supplemented animals showed a significant response. Slater confirmed their findings on riboflavin-deficient rats but suggested that, since the deficient animals ate so poorly, other nutritional deficiencies might account for the abnormality. This suspicion was justified by the fact that his pair-fed control animals receiving 0.1 mg. riboflavin per gram of diet developed the same impaired response to stress as the riboflavin-deficient animals, in disagreement with the findings of Slater and Morgan.

Certain differences, however, between the two sets of experiments might account for this discrepancy. Forker and Morgan applied a more drastic form of stress, namely anoxia, and the animals were given a deficient diet for 11 weeks and starved for 16 hours prior

to testing. Slater's group of animals were fed a similar diet, but the stress of unilateral adrenalectomy was less drastic and the animals were not in a fasting state. The inclusion of a group of animals fed a high-protein diet appears to eliminate a deficiency of amino acids as a limiting factor in the synthesis of either corticotrophin or adrenal cortical hormones. However, it seems apparent that the limitation of total diet did impair the pituitary-adrenal axis sufficiently to produce a measurable change compatible with a simple effect of starvation or with a deficiency of an unidentified factor.

The fact that corticotrophin was capable of depleting the adrenal gland of ascorbic acid, even though the animals were deficient in riboflavin, would tend to indicate impairment of release of the pituitary hormone. However, severe stress such as extensive laparotomy resulted in significant depletion of adrenal ascorbic acid. Slater considers the possibility that severe stress resulted in release of epinephrine or some other humoral agent, although he considers it more likely that the hypothalamic-pituitary system was activated to release corticotrophin.

This report emphasizes the difficulty of assessing a single physiologic response to depletion of an essential factor from the diet. It further emphasizes the need for meticulous control in nutritional studies and for the inclusion of pair-fed animals in any experiment which may alter the appetite of the subjects.

## NOTES

### Mannose Toxicity in the Honeybee

Mannose, a well utilized sugar by mammalian species, is metabolized first by being converted to the mannose-6-phosphate using adenosine triphosphate as a phosphorus donor. This is then followed by a second reaction which converts mannose-6-phosphate into fructose-6-phosphate, a common intermediate for glucose, fructose and mannose metabolism. The initial reaction of phosphorylation is catalyzed by hexokinase, an enzyme which performs this reaction for glucose, mannose and fructose. The second reaction is catalyzed by phosphomannose-isomerase.

Many years ago it was reported that the sugar mannose was very toxic for honeybees. Recently this problem was reinvestigated by A. Sols, E. Cadenas and F. Alvarado (*Science* **131**, 297 (1960)). These investigators studied three groups of 20 worker honeybees, allowing each group to feed on one of the following liquids: molar mannose, molar glucose solutions or water. Those fed the mannose had a 50 per cent mortality within one and one-half hours and over 90 per cent were dead in three hours. Of the controls receiving water, 90 per cent were dead within 12 hours, while 90 per cent of those fed the glucose solution were still surviving within three hours after the feeding period.

These investigators determined the hexokinase and isomerase activity in homogenates of whole bees killed by freezing at  $-20^{\circ}\text{C}$  after a one-hour fast. It was found that the homogenates of the honeybees had a high activity for the phosphorylation of glucose, mannose and fructose and very little phosphomannoseisomerase activity. The authors believe that mannose competitively inhibits both glucose and fructose phosphorylation and gives rise to an accumulation of mannose-6-phosphate.

It appears that the mannose toxicity occurring in honeybees can be considered a

metabolic disease resulting from a lack of balance between hexokinase and phosphomannose-isomerase activity. Thus honeybees have developed metabolic schemes whereby they can handle large amounts of glucose or fructose, but they do not have a mechanism for converting mannose-6-phosphate to a utilizable form of carbohydrate. These investigators suggest that the effect of mannose in the honeybee is not widely different from that of galactose in patients with galactosemia, although of course different enzyme systems are involved.

### Toxicity of Saturated Monobasic Aliphatic Acids and Their Esters

Technologic progress has increased the chances of man's exposure to many chemical substances. This is especially true of the aliphatic acids and their esters. Many of these compounds occur naturally in foods, and some may contaminate foods through their presence in insecticides, preservatives, solvents and dyes.

In two articles, W. F. von Oettingen (*A. M. A. Arch Indust. Health* **21**, 28, 100 (1960)) has compiled extensive information about those acids with two to 18 carbons. The report is factual and encyclopedic in style. It describes the chemistry, uses and irritant action of each acid, its manner of absorption, fate and excretion, pharmacologic or toxic effect, pathologic changes, prophylaxis and treatment of the toxic action.

These papers provide an important and ready source of reference which should be available to nutritionists, chemists and physicians.

### Physiologically Active Amines in Common Fruits and Vegetables

The content of serotonin and of catechol amines in bananas was reported recently (*Nutrition Reviews* **17**, 284 (1959)). It was concluded that ingestion of large amounts

of this fruit could lead to abnormal quantities of 5-hydroxyindole acetic acid and catechol amines in the urine of man. Recently R. W. Ewer, J. A. Arkins, B. T. Heffernan and E. J. Lennon (*J. Clin. Endocrinol. Metab.* **19**, 1037 (1959)) reported finding abnormal amounts of catechol amines in the urine of unselected hospitalized patients. Higher rates of excretion were found in patients with liver disease, infection of some types, psychotic reactions, or in those near death.

In further studies, fruits and vegetables were analyzed for their content of physiologically active amines (S. Udenfriend, W. Lovenberg and H. Sjoerdsma, *Arch. Biochem. Biophys.* **85**, 487 (1959)). Each fruit or vegetable was homogenized, centrifuged and assayed for content of 5-hydroxytryptamine, tryptamine, tyramine and catechol amines.

Serotonin was found in high concentration in banana skin and in lesser amounts in the pulp. Green bananas contained less than did ripe ones. Pulp of plantain, a tropical fruit, contained even more serotonin than did that of bananas. Tomatoes, plums and avocados contained significant quantities.

Tryptamine was found in plums, and tyramine was present in bananas, avocados, oranges and plums. Dopamine was intensely concentrated in banana peels, but only small quantities were present in banana pulp and in avocados. Norepinephrine was distributed in a similar fashion being concentrated in banana peel. Small quantities were found in banana pulp and in potatoes.

Amino acid decarboxylation is not limited

to a few plants, and may have important application since these substances could contribute to browning or discoloration of fruits. For example, certain amine oxidase inhibitors can prevent browning of banana skins.

The authors considered the presence of serotonin and catechol amines in these foods to be of no physiologic importance, since large quantities of these chemicals can be fed to man without adverse reactions. Apparently they are destroyed almost as rapidly as they are absorbed. The only clinical factor of significance would be the effect on urinary excretion of amines, which might lead to an erroneous diagnosis.

### Dietary Aspects of Cardiovascular Disease

The Heart Disease Control Program of the Division of Special Health Services, Department of Health, Education and Welfare, Washington, D. C., has just issued a publication (PHS OM-1175) titled *Dietary Aspects of Cardiovascular Diseases, Selected References*. The publication has been prepared chiefly for health department professional personnel needing a guide to current information and teaching materials on this subject. It may be useful also to private physicians, professional personnel in voluntary health agencies and hospitals, research workers, and other professional groups.

Beside general information, references are given to material on calorie restriction (weight control and obesity), sodium restriction (congestive heart failure and hypertension), fat control (atherosclerosis and coronary artery disease), and to food composition tables in current use.



# NUTRITION REVIEWS

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## ABERRANT LIPOGENESIS

The formation of microscopically visible fat by cells other than adipose tissue is called aberrant lipogenesis. This article outlines our experiments and interpretations of this process, summarizing papers published over the past several years. Although studied first and most extensively in the cornea, aberrant lipogenesis is a widespread phenomenon with, possibly, particular relevance to fatty degeneration and atheromatosis. It is a dynamic process of fat synthesis, culminating in glyceride esters, clearly distinguishable from phagocytosis of preformed fat (lipophanerosis).

Aberrant lipogenesis may be demonstrated *in vivo* by the injection of oleic, palmitic, or stearic acid into suitable tissues, but it is best induced by incubation of surviving tissue at 37°C in media containing salts of these fatty acids together with appropriately buffered serum. The fatty acid concentration (2 to 5 mg. per ml.) is critical; several-fold dilution will yield insignificant fat while excess will kill the tissue. The hydrogen ion concentration must be about neutrality (pH 6.8 to 8.0). The cornea is an especially favorable tissue because it has a relatively simple structure and yet contains components of epithelium, connective tissue and endothelium.

Incubation of corneal pieces in suitable media containing sodium oleate results in the formation of sudanophilic globules that appear in the cytoplasm first at six hours and increase progressively for several days. Through coalescence the globules eventually attain grotesque proportions several times the size of the host cells. All the cells of the cornea (epithelium, stroma and endothelium) participate in this lipogenesis, yielding consistently reproducible results with successive experiments. If palmitic or stearic acid

is substituted for oleic acid, the cells synthesize a lipid having the same distribution and the same time sequence but having a birefringent crystalline structure rather than sudanophilic globules.

With labeled fatty acids, it has been shown that the lipid formed is a neutral glyceride. Whether this product is mono-, di-, or triglycerides, or what proportion of each, has not yet been established.

Serum is essential to the process of aberrant lipogenesis. The precise role played by serum is not understood but both dialyzable and one or more non-dialyzable components are necessary. Calcium and magnesium may replace the former, but no one component (Cohn's fractions, lipoprotein, albumin, etc.) has been satisfactorily substituted for the latter. The serum factor is thermostable under conditions short of those which will coagulate the protein.

No significant accessory factors have been discovered that enhance the lipogenesis. Nucleotide co-factors and adenosine triphosphate have no effect when added to the medium. Addition of glucose and insulin or glycerol is similarly without effect. Cholesterol does not induce lipogenesis and does not increase that which occurs when sub-optimal amounts of fatty acids are added (contrary to what has been found by others for tissue culture preparations). Occasionally hypercholesteremic serum from patients or rabbits will induce a small amount of fat formation, but this appears to be due to the increased availability of fatty acids rather than to the cholesterol.

Aberrant lipogenesis is an enzymatic and respiratory-dependent process. It is inactivated by prior heating of the tissue to 70° and is inhibited by para-chloromercuribenzoate, arsenite, or iodoacetic acid. It is sig-

nificantly diminished by sodium fluoride and potassium cyanide. Moreover, it appears to be a unidirectional process. There is no breakdown of the fat formed *in situ* and the lipid, when extracted and reinjected into the cornea *in vivo*, acts like a neutral fat that will be disposed of slowly by phagocytosis but will not induce lipogenesis. Yet to be studied is lipogenesis with separate sub-cellular particles. Identical results have been obtained with tissue from all animals tested (rabbit, cat, beef, mouse and man).

The tissue most profitably studied has been the cornea, but many tissues throughout the body are capable of aberrant lipogenesis. In general, the two types of tissue response are: (1) that typified by the cornea in which addition of fatty acids is mandatory for lipogenesis, and (2) that typified by the liver in which sufficient endogeneous substrates (presumably fatty acids) are present in the tissue to provide a built-in substrate for lipogenesis. The former type includes most connective tissue, arteries, and some epithelial membranes while the latter type includes parenchymatous organs such as the liver and kidney. Some tissues such as spleen, lymph nodes, white blood cells and adipose tissue (from starved animals) are not capable of aberrant lipogenesis under the experimental conditions.

A further difference between the tissues requiring the addition of fatty acids and those not requiring it is that the parenchymatous tissue, such as the liver, shows only a marginal lipogenesis on incubation of excised pieces (due to failure of the deeper cells to survive) whereas the other type of tissue, exemplified by the cornea, shows uniform lipogenesis throughout the entire tissue.

The foregoing experimental observations would appear to have a direct bearing on the origin of fat in so-called fatty degeneration and possibly in atherosclerosis. Fatty degeneration consists in an accumulation of fat within viable cells on the edge of a necrotic lesion. Focal lesions of the liver induced *in*

*vivo* show this rim of sudanophilia well. The fat forms promptly in the surviving cells but may be prevented from doing so by the addition of para-chloromercuribenzoate to the necrotizing agent.

A more direct visualization of lipogenesis from necrotic tissue may be obtained by injecting liver brei into the cornea. Fat will then form in the adjacent corneal cells similar to that which occurs when fatty acids exclusively are injected into the cornea. It will not occur if the lipid is first extracted from the liver brei. There would appear to be little doubt, therefore, that in this analogue of fatty degeneration, it was the liberated fatty acids of the necrotic liver which were inducing the lipogenesis in the corneal cells.

A similar process is believed to underlie the fat formation in atheromatosis, but crucial experiments to prove this have yet to be done. The question is, of course, why blood vessels and other tissue do not form fat abundantly during life, since it is obligatory for them to do so experimentally when exposed to native fatty acids and serum. A partial answer may be in the availability (or unavailability) of the fatty acids or, in other words, in the stability of the protein complexes that normally bind the fatty acids and may prevent their utilization by the cells of the body.

Of perhaps greater biologic significance than either fatty degeneration or atheromatosis is the usefulness of aberrant lipogenesis to the body economy. Free fatty acids are potent necrotizing agents and it would appear imperative to have a mechanism for promptly converting those which become available into an innocuous form. In the blood stream there is abundant evidence that physiologic quantities of fatty acids are bound promptly to albumin and thereby detoxified, but in the tissue the mechanism appears to be one of rapid esterification into a neutral and, therefore, innocuous triglyceride. In this form, it may either remain indefinitely as an inert fat reservoir or it

may be disposed of by the leisurely process of phagocytosis. According to this concept, which is admittedly speculative, aberrant lipogenesis serves a biologically useful function in protecting the body from one of its own, potentially toxic, constituents.

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### "THERAPY" WITH VITAMIN E

*Despite many claims to the contrary, convincing evidence is lacking that vitamin E is effective in treating threatened abortion or muscular dystrophy of unknown etiology.*

A report, "Should Spontaneous Abortion Be Prevented," by E. V. Shute has appeared recently (*Canad. Med. Assn. J.* **82**, 72 (1960)). As the title suggests, the article is biased. For many years Shute has published accounts of the therapeutic efficiency of vitamin E in such diverse conditions as pruritis vulvae, rheumatic fever, angina pectoris and myocardial infarction, infertility, threatened abortion, nephritis, and pre-eclampsia (A. V. Vogelsang and E. V. Shute, *Nature* **157**, 772 (1946); *Nutrition Reviews* **5**, 164 (1947); **7**, 118 (1949); Vogelsang, E. V. Shute and W. E. Shute, *Medical Record* **160**, 279 (1947); **161**, 155 (1948)). Unfortunately, studies conducted by others have failed to confirm his claims.

A brief reappraisal of the history of vitamin E will aid in an understanding of the problem. An essential milk factor was studied in 1920 and found to be essential for the growth of animals (H. A. Mattill and R. E. Conklin, *J. Biol. Chem.* **44**, 137 (1920)). Soon this substance was found to be necessary for reproductive processes as well as growth in animals. It was found in such vegetable foods as lettuce, peas, several cereals and particularly in wheat germ. It was also found in beef liver and in egg yolk (H. M. Evans and K. S. Bishop, *J. Am. Med. Assn.* **81**, 889 (1923)). At the same time, scientific curiosity in the potential curative effects of other vitamins was

growing rapidly and soon a number of reports appeared relating to vitamin E. The active substance was found to be a tocopherol which later was divided into three fractions: alpha, beta and gamma, having a relative activity in that order. An interesting chemical effect of these fractions was the protection of other fats from oxidation, but here their relative effectiveness was in reverse order (gamma, beta, alpha).

Meanwhile deficient states were induced in many species of animals (Evans and G. O. Burr, *J. Biol. Chem.* **76**, 273 (1928); H. S. Olcott, *J. Nutrition* **15**, 221 (1938); C. G. Mackenzie and E. V. McCollum, *Ibid.* **19**, 345 (1940)), and others. Lack of this vitamin resulted in such abnormalities as a muscular dystrophy with creatinuria, peculiar lesions of the central nervous system, cardiac failure, pulmonary hemorrhage, testicular degeneration and absorption of gestational products in rats. Pathologic studies of muscular lesions disclosed a peculiar type of myositis which was accompanied by ceroid deposits in many instances (K. E. Mason and A. F. Emmel, *Anat. Record* **92**, 33 (1945)). Muscle tissue from deficient animals was found to have an abnormally high rate of oxygen consumption (O. B. Houchin and Mattill, *J. Biol. Chem.* **146**, 309, 313 (1942)), a finding which led to the observation that the vitamin has an anti-oxidation effect.

These and many other examples of ac-

curate investigation gave ample reason for clinical investigators to consider the possibility that vitamin E might have some curative or beneficial effect in human disease which resembled, even slightly, those induced in animals. Unfortunately uncritical evaluation of results led to a few initial reports which were unjustifiably enthusiastic. In every instance of scientifically planned and controlled studies the results of therapy with vitamin E have shown no significant effect (except in the rare instance of certain types of biliary disease).

In this most recent report, Shute (*loc. cit.*) describes the results of the administration of alpha-tocopherol (exact dose not stated) to 232 women who had "threatened abortion . . . (or) threatened miscarriages." No evidence is given for classifying these pregnant women in the above categories, but 195 (84 per cent) delivered children. Thirteen of these had congenital anomalies. There were no control subjects for comparison, and no other forms of treatment were tried. Thus there would seem to be no reason to believe that the medication had any effect.

A somewhat more objective report was described by G. M. Berneske, A. R. C. Butson, E. N. Gauld and D. Levy (*Canad. Med. Assn. J.* **82**, 418 (1960)), who reported their observations of 33 patients with muscular dystrophy. The group included 16 boys with pseudohypertrophic juvenile dystrophy, one girl with a non-hypertrophic juvenile dystrophy, and 16 patients of both sexes with a facio-scapulo-humeral or limb-girdle type of muscular dystrophy. The patients ranged from six to 58 years of age and had been observed for significant periods of time prior to the study. Treatment consisted of the oral administration of 2000 international units of alpha-tocopherol succinate to 22 of the patients and a suitable placebo to the other 11. Careful records were made of the strength of various muscle groups. At the close of the study when the patients had been given these medications

for periods of 11 months to three years, muscular strength was again evaluated and recorded.

The data indicated that there was no difference between the effect of alpha-tocopherol and that of the placebo. The experimental design, although imperfect, did serve to demonstrate this lack of effect. The study would have been of more value if the vitamin and placebo had been given to the patients according to the double blindfold technique and if the groups receiving each of these had been equal. Similarly, it would have been preferable to have given the treatment for the same length of time to each patient. Also it would have been helpful to have studied the urinary excretion of creatinine, but the negative effects seem sufficiently evident. Unfortunately, the authors made the statement that the results were "sufficiently indecisive, so that we feel that we should not draw dogmatic conclusions."

There can be no doubt that vitamin E is an essential in the human economy and instances of true deficiency have been reported. One such patient with xanthomatous biliary cirrhosis was described by C. W. Woodruff (*Am. J. Clin. Nutrition* **4**, 597 (1956)). Similar deficiency was reported by H. M. Nitowsky, H. H. Gordon and J. T. Tildon (*Bull. Johns Hopkins Hosp.* **98**, 361 (1956)). In these instances, infants who were suffering from cystic pancreatitis and associated congenital biliary atresia had low serum levels of tocopherol and had creatinuria. A recently described test evaluating the susceptibility of erythrocytes to hemolysis by hydrogen peroxide (C. S. Rose and P. György, *Am. J. Physiol.* **168**, 414 (1952)) allows the investigator to detect more subtle degrees of vitamin E deficiency.

With the abundance of information available as to the chemical nature and the several functions of this vitamin and with knowledge of methods for detecting its concentration in the serum (its protection of erythrocytes

from undue hemolysis and its effect upon the urinary excretion of creatinine), it is surprising to find that clinical investigators persist in the unworthy method characterized by administration of a substance to patients with a given disease and willingness to ascribe any coincidental change to an

effect of that substance. Equally regrettable is the willingness of editors of medical publications to accept uncritical reporting for publication. This has led to general discredit of vitamin E as well as to the necessity for frequent studies which result in negative reports.

## HEPATIC COMA

*Neurological symptoms accompanying severe liver disease may be controlled by emptying the colon, feeding a protein-free diet, and giving antibiotics to prevent putrefaction.*

In patients suffering from advanced hepatic disease, neurologic disorders are common. These take the form of mental confusion, flapping tremor, impaired consciousness, coma and death.

Many attempts have been made to explain this syndrome commonly called hepatic coma. Experiments in dogs have demonstrated that shunting of the portal venous blood into the inferior vena cava (Eck fistula) results in an inability of the animals to tolerate much dietary protein. When fed meat, they develop strange behavior and may become comatose. Apparently bypassing the liver prevents normal metabolism of amino acids. Both experimental animals with an Eck fistula and cirrhotic patients have abnormal concentrations of ammonia in their blood after eating protein, although the correlation between levels of ammonia and state of consciousness has been imperfect. Most investigators, however, feel it is an important factor in production of hepatic coma.

Efforts have been made to decrease the quantity of ammonia in the blood. Glutamic acid promotes utilization of ammonia in the brain (*Nutrition Reviews* 15, 314 (1957)), and arginine participates in the urea cycle whereby carbon dioxide and ammonia are converted into urea. Another amino acid, methionine, has been found to induce neurologic symptoms in patients with hepatic

cirrhosis (*Ibid.* 14, 336 (1956)), although the mechanism is not clear.

Attempts at treatment of this disorder have been partially successful and restriction of dietary protein seems to benefit many patients (*Nutrition Reviews* 14, 74 (1956)). Another method depends upon oral administration of antibiotics (tetracycline or neomycin) to lessen the degree of intestinal putrefaction of food (A. M. Dawson, J. McLaren and S. Sherlock, *Lancet* II, 1263 (1957)). Although most clinicians have abandoned the use of glutamic acid or arginine, a few continue to find the latter helpful (J. L. Fahey, D. Nathans and D. Rairigh, *Am. J. Med.* 23, 860 (1957)).

G. C. Willis has reported his experiences in managing 120 cases of hepatic coma or impending coma (*Canad. Med. Assn. J.* 82, 191 (1960)). All of these had demonstrable liver disease; 100 had cirrhosis, 11 had acute hepatic necrosis, and nine had hepatic malignancy. By way of comparison, the author studied 100 other patients with cirrhosis who did not have coma or mental symptoms.

Of the 120 patients, 88 had flapping tremor, 86 had actual coma, and 80 were visibly jaundiced. Feter hepatitis was noted in 28 per cent of the group with coma and in 25 per cent of those without. Factors which might have precipitated the onset of coma included gastrointestinal hemorrhage,

pneumonia, abdominal paracentesis and sedative drugs.

Treatment was as follows: correction of the precipitating factors, administration of magnesium sulfate and an enema, feeding a protein-free diet of 2000 calories, and oral administration of 250 to 500 mg. tetracycline every six hours. Seventy-eight patients survived. The prognosis was best in those with cirrhosis (74 per cent) and poorest in those with acute hepatic necrosis (9 per cent).

The author explains his reasons for believing that hepatic coma is caused by parenchymal cellular damage rather than by portal hypertension with secondary anastomoses. In the 100 cirrhotic patients without coma, the incidence of visible collateral circulation was greater (27) than in those with coma (25). Similarly, splenomegaly and ascites (additional signs of portal hypertension) were less common in cirrhotic patients with coma than in those without. The poor prognosis in those patients with acute hepatic disease would support his contention. Feto hepaticus was thought to result from products of intestinal putrefaction and was observed only once in the control group, but 25 times in those with coma.

Of the factors which may precipitate coma, gastrointestinal hemorrhage was most important. This might be due to hypotension, which reduced hepatic circulation, and to the increased putrefaction of blood. Ingestion of blood results in a greater rise of blood ammonia than ingestion of other proteins in like amount (A. N. Bessman and G. S. Mirick, *J. Clin. Invest.* **37**, 990 (1958)).

Neither the exact cause of hepatic coma nor the ideal form of treatment has been found. The patient with this condition has many coexisting biochemical defects. Perhaps the abnormal concentration of ammonia in the blood, the loss of potassium from cells, as well as the toxic effect of products of intestinal putrefaction are all important factors in the production of this syndrome. S. Sherlock has employed a form of therapy similar to that of Willis (Sherlock, W. Summerskill, and A. Dawson, *Lancet* **II**, 689 (1956)), but she used neomycin and, in addition, gave hydrocortisone to patients with acute hepatic necrosis.

Without the accouterments of a research grant, a biochemical laboratory or even the use of statistics, Willis has made a significant contribution by simply observing his patients and recording what he saw.

### AMINO ACIDS IN PLASMA AND URINE IN KWASHIORKOR

*Children with kwashiorkor were found to have an aminoaciduria. Also ethanolamine and beta-aminoisobutyric acid were found in the urine. The latter two substances disappeared upon feeding an adequate diet.*

Kwashiorkor is a disorder caused by decreased protein intake. This means that the limiting nutrients are specific amino acids. It has been reported that better nitrogen balance occurs in individuals with kwashiorkor when they receive a mixture of 18 amino acids (including all the essential ones) than when they receive just the essential amino acids alone (*Nutrition Reviews* **15**, 132 (1957)). Recently, J. C. Edozien, E. J. Phillips and W. R. F. Collis (*Lancet* **I**, 615

(1960)) have studied the free amino acids in plasma and urine of children with kwashiorkor.

These investigators observed 50 new untreated cases of kwashiorkor in children between the ages of one and four years. On admission they were kept on glucose and water with added potassium until the following day when the fasting blood and urine specimens were obtained. After these initial samples were taken, 41 of the children were



treated with a high-protein milk diet either as out-patients or in the hospital. The nine remaining individuals, none of whom was seriously ill or in evident danger, were studied on different regimens. Three of the children were fed for four days a diet high in carbohydrate but low in protein (calculated to contain less than five g. of protein per day). The other six were given identical diets to which was added 2 g. of L-methionine per day. At the end of this four-day period both groups were then placed on a high-protein diet. For controls, the investigators used 25 children of the same age range.

The authors found the average fasting plasma amino acid nitrogen level for healthy children to be 5.2 mg. per 100 ml. (4.1 to 8.0 mg.) if the method of Folin was used, and 4.3 (3.5 to 6.1) with the ninhydrin procedure. For patients with kwashiorkor, the plasma amino acid nitrogen levels were uniformly lower, being 2.5 mg. per 100 ml. (2.0 to 4.5) and 2.1 mg. per 100 ml. (1.3 to 3.5 mg.) by the two methods, respectively. Thus the plasma level of amino acid nitrogen appeared to be about 45 per cent less than that found in comparably healthy children of this area. A decrease in intensity of ninhydrin reacting spots appeared in the plasma of the patients with kwashiorkor, although this was not a uniform reaction. The amino acids which appeared to be most affected were cystine, methionine, valine, tryptophan, leucine, isoleucine and phenylalanine, although these procedures were only qualitative. In the chromatograms obtained from plasma of normal individuals ethanolamine could not be detected, but was clearly visible in the plasma obtained from 75 per cent of the patients with kwashiorkor. Also beta-aminoisobutyric acid could not be found in the blood of the controls, but was found in 30 per cent of the children with kwashiorkor.

In order to study the amino acid content of urine, these investigators used uniformly 50 microliters of urine without regard for

volume or period of collection. Thus the excretion values are at most only semi-quantitative results, and offer no data on the total daily excretion of amino acids. Ethanolamine was not found in any of the normal urines but beta-aminoisobutyric acid was detected in 8 per cent of the urine samples obtained from normal individuals. However, ethanolamine was excreted in the urine of 80 per cent of the patients with kwashiorkor and beta-aminoisobutyric acid was identified in the urine in every case. In many of the pathological urines this amino acid produced a dominant spot. It should also be noted that the essential amino acids could be identified only rarely on the chromatograms obtained from the urine of the patients with kwashiorkor; most of the amino acids present were the nonessential acids.

On adding methionine to the diet of the patients with kwashiorkor, there was a marked increase in all plasma amino acids. In three individuals in whom ethanolamine was initially present in the plasma, the ethanolamine disappeared after methionine supplementation. Although the ethanolamine in the urine was greatly diminished in all six cases, the amount of beta-aminoisobutyric acid was not affected by the administration of methionine.

These investigators believe that the aminoaciduria in kwashiorkor is of the renal type since there is increased excretion of amino acids in spite of the level of these compounds in the plasma. Although urine analysis rarely shows any specific urine abnormality and no specific lesion in the kidney has been described, these investigators believe that the renal tubular cells are suffering from a generalized loss of cellular contents including, among other proteins, specific cellular enzymes. As such, the aminoaciduria could be the result of a defect in the reabsorption mechanism of amino acids by the renal tubules.

The beta-aminoisobutyric acid probably has thymine as its precursor. It is believed that the large amounts of this unusual amino

acid may be derived from cellular desoxy-ribonucleic acid, which is released by the degradation of skeletal muscle and organ tissues. While ethanolamine has been known to be excreted with other amino acids in cases of liver disease, these investigators could find no constant relationship between the plasma ethanolamine and ethanolamine-uria on the one hand and the results of various liver function tests on the other, although again it should be noted that the urine collections were not standardized

periods. The high level of ethanolamine in plasma may be due to a metabolic block in which ethanolamine cannot be utilized in other synthetic processes.

While the generalized nutritional deficiency in kwashiorkor is well known to be a protein defect, studies of a type presented by these investigators provide information which later may reveal the mechanisms by which a protein deficiency produces such a wide spectrum of changes.

### REFRACTORY RICKETS AS A SEX-LINKED CONGENITAL ANOMALY

*Rickets resistant to vitamin D therapy appears to be a sex-linked dominant familial hypophosphatemia. Affected males show more intense lesions than females, but only daughters of affected males show hypophosphatemia.*

The occurrence of rickets resistant to vitamin D therapy has been recognized (F. Albright, A. M. Butler and E. Bloomberg, *Am. J. Dis. Child.* **54**, 529 (1937)), and the use of massive doses of vitamin D has been necessary to produce healing of the bone lesions in such instances. It has been suggested that the type of rickets which is refractory to dietary supplements of vitamin D results from a congenital or acquired metabolic defect.

J. B. Graham, V. W. McFalls and R. W. Winters (*Am. J. Human Genetics* **11**, 311 (1959)) have recently studied large enough groups of related individuals to conclude that this condition is not a rare and exotic entity. These authors have characterized this condition as a sex-linked dominant familial hypophosphatemia, and their study of the clinical and chemical conditions associated with this disease represents an extension of an earlier report on a different family group (Winters *et al.*, *Medicine* **37**, 97 (1958)).

Study of each instance of refractory rickets (as evidenced by bow legs, knock-knees, or the onset and progression of characteristic skeletal deformities despite vitamin D therapy, or by the typical radiographic signs

of rickets in children or post-rachitic deformities in adults) led to identification of a kindred type exhibiting familial hypophosphatemia.

Hypophosphatemia was described by J. C. Rathbun as a developmental anomaly (*Am. J. Dis. Child.* **75**, 822 (1948)), and Graham, McFalls and Winters (*loc. cit.*) have pointed out that it is only on the chemical evidence of low serum inorganic phosphorus that a genetic relationship can be detected. In order to detect abnormal serum inorganic phosphorus values in children, it was necessary to set up normal ranges by fitting regression curves by age and sex for a large normal population and using the 99 per cent confidence limit as a dividing point. Blood serum inorganic phosphorus values which fell below this limit were considered to be hypophosphatemic.

It was quite evident that bone deformities were more likely to occur in hypophosphatemic males than in affected females. On the other hand, daughters of affected men were uniformly hypophosphatemic while in no case did the son of an affected man have the condition. In the case of affected females, their progeny approached a ratio of 1:1:1:1 (affected males: normal

males: affected females: normal females), which would be expected of a sex-linked dominant character.

The problem of penetrance was considered by Graham, McFalls and Winters and they concluded that the hypophosphatemia is almost, if not completely, penetrant. The bone disease is far less so and is affected by a sex difference in the expression of the abnormal gene. Of males showing hypophosphatemia, 93 per cent showed bone disease, but only 25 per cent of the females exhibited bone changes. In addition, the severity of the bone lesions appeared to be more intense in males, with females having only moderately severe lesions.

These investigators have considered at some length the possibility that males may have a more depressed serum inorganic phosphorus value than females. Certainly the values presented indicated a mean value lower by 0.46 mg. per cent for the males. However, a careful evaluation of the values and associated factors of age and environment suggest that perhaps this difference

should be viewed with caution until more complete studies of larger numbers of individuals can be made.

With respect to nutrition, it is of interest that the group involved in this study were predominantly from farm families who customarily consumed large quantities of dairy products and were generously exposed to sunlight. In practically every instance, individuals who showed signs of rickets as young children were given generous supplements of cod-liver oil. A low serum inorganic phosphorus value was consistently observed. This blood abnormality, however, was not consistently related to bone deformities, especially in females.

The observations of Graham, McFalls and Winters certainly emphasize the genetic basis for a rather common occurrence of refractory rickets. The almost complete elimination of dietary rickets from the United States should suggest that occurrence of rickets resistant to vitamin D therapy ought to be viewed as a possible congenital anomaly.

## CARBOHYDRATE METABOLISM DURING ETHER ANESTHESIA

*Without surgical trauma, ether anesthesia alone will induce changes in glucose metabolism as indicated by decreased glycose tolerance, increased inorganic phosphorus levels and changes in pyruvate concentrations of the blood.*

The metabolic effects of trauma have been reviewed earlier (*Nutrition Reviews* 16, 196 (1958)). However, in studying the changes induced in patients following surgical trauma, it is necessary to separate the effects due to the surgical procedure from those caused by the anesthetic used in the operation. Ether (diethyl ether) is one of the common anesthetic agents used in surgical procedures and is usually used in conjunction with other substances such as nitrous oxide and barbiturates (thiopental sodium).

Knowledge that ether anesthesia induces metabolic changes in man is not new. A. Reynoso (*Compt. Rend. Soc. Biol.* 5, 116 (1853)) noted sugar in the urine of patients

who were anesthetized with ether. This was less than ten years after the discovery of ether anesthesia. Over a period of years it has been frequently reconfirmed that ether anesthesia induced an elevated blood sugar level and frequently glycosuria.

Recently, a team of investigators led by W. R. Drucker (*Metabolism* 8, 827 (1959)) studied the effects of ether anesthesia on glucose and fructose metabolism by performing three intravenous glucose tolerance tests on each of six patients and three intravenous fructose tolerance tests on each of another six patients. The first (control) test was performed a day or two before the patient was anesthetized. The second test was

performed on the day of anesthesia including the period when the ether was being given, and the third test on the day following the anesthesia. No operations were performed at this time and the period of anesthesia was 90 minutes. The volunteers used in this study had been hospitalized for the rehabilitation of a nonprogressive illness several weeks before the study was undertaken.

The hexose tolerance tests were carried out by giving 1 g. of either glucose or fructose per kilogram of body weight per hour, in the form of a 10 per cent sugar solution. All infusions lasted one hour. Samples of venous blood were drawn at the beginning of each study and at 30-minute intervals for a total of three hours, and the levels of glucose, fructose, pyruvate, lactate and inorganic phosphorus were determined.

Such studies require a high degree of control if one wishes to eliminate numerous extraneous factors which would mask or confuse the metabolic aspects of anesthesia. These investigators believed that they incorporated three important features which increased the degree of control. The first was a fully equipped operating room in which to carry out all the studies. Thus familiarity with the environment tended to reduce anxiety on the day of anesthesia. Secondly, the anesthesia was given to all of the subjects by the same trained anesthetist. This eliminated variation in anesthetic procedures. Finally, no surgery was performed and thus the metabolic effects of anesthesia were not complicated by the changes induced by surgery.

Anesthesia alone caused a rise in the blood glucose levels along with an increase in lactate and inorganic phosphorus concentrations in the serum. The amount of fructose and pyruvate present in the blood did not change when ether was given.

When glucose tolerance tests were carried out on patients receiving ether, the glucose levels of the blood were elevated as compared to the control period of study. However, the decay rate of the glucose tolerance

curve did not differ from that observed when the patient received no ether. No changes in the fructose tolerance curves were noted upon administering ether. However, there was an increased level of glucose in the blood following the fructose administration. Thus when glucose is formed from fructose in the body it cannot be utilized as well as the original fructose in the anesthetized patient.

A small rise in the pyruvate levels in the blood was noted after giving glucose, and a greater rise occurred if the patient was anesthetized. Fructose without anesthesia induced blood levels of pyruvate which were much higher than in the anesthetized patients receiving glucose. However, on giving ether to patients receiving fructose, smaller rises in the pyruvate levels were noted when compared to the non-anesthetized individual. On the day following ether, the fructose tolerance test caused a rebound in the pyruvate levels resulting in greater values than during the control period.

It is well known that during either a glucose or fructose tolerance test in a non-anesthetized individual there is a fall in the level of inorganic phosphorus in the blood, indicating that organic phosphorus compounds are being formed. However, in the present study in the individuals anesthetized with ether there was an initial rise in the inorganic phosphorus levels, which continued despite the administration of glucose. On the other hand, when fructose was given to an anesthetized patient this early rise was reversed and there was instead a decrease in inorganic phosphorus during the first 30 minutes of sugar infusion. Thereafter, however, the inorganic phosphorus level increased abruptly despite continued fructose administration. The initial fall in inorganic phosphorus on giving fructose intravenously also supports the view that fructose can be utilized by the anesthetized individual.

It has been thought previously that the changes in carbohydrate metabolism noted in the anesthetized patient are the result of

the secondary effects of increased adrenalin production induced by the ether. This group of investigators studied two individuals who were given sufficient adrenalin to cause their blood sugar to reach levels noted in patients given ether, and only a small rise in the blood pyruvate occurred along with a fall in the serum inorganic phosphorus levels. Also a series of studies was carried out with a patient who had a transection of the spinal cord high enough to preclude all central nervous stimulation to the adrenal medulla. On administering ether to this patient, no change in the pyruvate or rise in the inorganic phosphorus level was noted. Thus these two studies tend to rule out increased

adrenalin production as the only causative agent in the metabolic changes induced by ether anesthesia.

On the day following the anesthesia, all of the metabolic changes that had been noted in carbohydrate metabolism disappeared. This is in contrast to the changes in carbohydrate tolerance observed in post-operative patients, which may last many days. The changes found following ether anesthesia are consistent with changes noted in a steroid type of diabetes, but the relationship between these two phenomena are not known. A fuller explanation of the cause of the abnormality in carbohydrate metabolism induced by ether is not yet available.

## ACCUMULATION OF INSECTICIDES IN TISSUES AND EXCRETION IN MILK

*Steers, swine, poultry and lambs show marked differences in the tissue accumulation of dieldrin. Both tissue accumulation and milk excretion levels are roughly proportional to the level in the feed.*

Milk and milk products make up such a large proportion of the diet of children and adults in the United States that any change in milk composition through changes in agricultural practice or processing techniques is of great interest to everyone. The use of modern insecticides to protect plants and animals from harmful insects led inevitably to studies of the accumulation of insecticide residues in animal tissues used for food and the excretion of these residues in milk.

It was early demonstrated that the chlorinated hydrocarbon insecticides were stored in animal fat in varying degrees. Consequently, the extent to which these insecticides may be used on forage crops or on animals depends greatly on their propensity for storage in the tissues of animals and upon the excretion of these insecticides in milk. Some insecticides have been assigned tolerance levels in the fat of certain animals, but, at the present time, regulatory agencies have maintained that

milk is not to be contaminated by pesticides or other foreign substances.

N. Gannon, R. P. Link and G. C. Decker (*J. Agr. Food Chem.* 7, 824, 826, 829 (1959)) in a series of three papers have investigated the storage of dieldrin in the tissues of steers, hogs, lambs, poultry and eggs, as well as the excretion of a number of insecticides (aldrin, dieldrin, D.D.T., heptachlor and methoxychlor) in milk of dairy cows fed these substances in the daily ration. Their results indicate that there are great differences in the way the different species handle dieldrin, but it is plain that the fat tissues accumulated dieldrin at all of the levels fed (0.1, 0.25, 0.75, and 2.25 p.p.m.). At the end of 12 weeks, the accumulation of dieldrin in the fat of the different species was roughly proportional to the level of insecticide in the diet.

Gannon, Link and Decker took great care to insure a quantitative intake of the

insecticide and, following a feeding period of 12 weeks, some of the animals and poultry were sacrificed to determine insecticide accumulation in the tissues. The remaining animals were retained on an insecticide-free diet to determine the rate of elimination over a period of six weeks. Their results demonstrate that tissue accumulation of dieldrin is directly related to the amount of fat in the diet.

There were distinct differences between species; steers stored about twice as much dieldrin as hogs and about five times as much as lambs. On the other hand, hens stored about ten times as much in their body fat as did steers, although they excreted a barely measurable amount in their eggs. In general, renal fat contained most insecticide, slightly exceeding the values obtained for body fat. This probably reflected the higher actual fat content of the renal fat tissues. In steers, roasts and steaks had the next highest storage values, although no dieldrin was measurable in these tissues when fed at levels of 0.25 p.p.m. Liver and kidney tissues stored the smallest amounts of insecticide.

Lambs showed no dieldrin in the chops over the entire range of dosages, and the level in the roast was probably not significant. Six weeks after dieldrin was discontinued, the fat of steers and hogs had dropped about 40 per cent while the fat of lambs had dropped about 70 per cent from the level attained after 12 weeks of feeding.

In dairy cattle, the level of dieldrin excreted in the milk was proportional to the level in the feed. When the cows were on a continuous intake of the insecticide, there appeared to be an approximate straightline relationship between p.p.m. of dieldrin in the milk and p.p.m. in the feed. By the end of the 12-week feeding period, dieldrin was present in the milk in measurable quantities at all levels of the insecticide feeding. As in beef cattle, dairy cattle tissues analyzed

for dieldrin at the end of 12 weeks' feeding showed accumulations of dieldrin roughly proportional to the amount of fat in the tissues and to the level of insecticide in the diet.

The work of Gannon, Link and Decker suggests that the ratio of dieldrin in milk to the dieldrin in the fat of the tissues when the cattle are on a daily dieldrin intake will range between 12 and 18 to 1. After six weeks on a dieldrin-free diet, it was evident that the dieldrin in milk had dropped by 80 per cent while that in the body fat had dropped by 30 per cent from the levels attained at the end of the insecticide feeding period.

Residues in milk resulting from feeding other chlorinated hydrocarbon insecticides, as well as dieldrin, were also determined. The levels fed were as follows: aldrin 1, 10, 40 p.p.m.; dieldrin 10, 50, 75 p.p.m.; heptachlor 50, 75, 100, 200 p.p.m.; D.D.T. 10, 25, 100, 200 p.p.m.; and methoxychlor 800, 1000, 4000, 7000 p.p.m. The results of these trials indicated that aldrin (excreted as dieldrin) was excreted roughly twice as readily as dieldrin itself, ten times as readily as D.D.T., 20 times as readily as heptachlor (excreted as heptachlor epoxide), and 1600 times as readily as methoxychlor. Storage in the body fat was of the same order of magnitude as secretion in the milk.

While it was apparent that there was some difference in rate of loss of the insecticides as compared to rate of storage when evaluated over a period of seven and one-half weeks following discontinuation of insecticides, methoxychlor was lost most rapidly and D.D.T. was lost much more rapidly than the remaining insecticides studied.

The results presented by these investigators in this series of three papers are of great interest to both the consumer and producer of meat, milk and eggs, since they emphasize



the different ways in which various species metabolize and handle a given insecticide. Certainly such work will provide a firmer

basis for evaluating the use of insecticides and establishing tolerance levels when these are used in animal feeds.

### WATER-SOLUBLE AND -INSOLUBLE GROWTH FACTORS IN DIETS CONTAINING TUMOR TISSUE

*The sodium ion content of tumor diets is responsible in part for increased food and water ingestion and carcass hydration in rats, but still other appetite and growth factors remain unidentified.*

Previous studies (*Nutrition Reviews* 17, 274 (1959)) have demonstrated that lyophilized tumor tissue used as a source of nitrogen in the diet can stimulate food intake and weight gain in tumor-bearing Sprague-Dawley rats that have reached a weight plateau on a 20 per cent casein diet. The details of experiments pointing to sodium ion as an agent responsible for this phenomenon but suggesting the presence of an additional water-insoluble factor in tumor tissue have been published by J. White, J. N. Toal, F. K. Millar, and R. H. Brooks (*J. Nat. Cancer Inst.* 24, 197 (1960)).

The tumor diet contained Walker carcinosarcoma 256 tumor tissue, which had been lyophilized and defatted by treating the lyophilized tumor tissue with 2:1 alcohol-ether followed by 1:1 chloroform-methanol. Additional alcohol-ether removed the residual chloroform. The lyophilized defatted tissue was extracted five times with boiling distilled water for periods of 20 minutes each with the use of 3.75, 1.5, 1.5, 1.5 and 1.0 liters per kg. for successive extractions. The residue was washed with alcohol and ether consecutively and air-dried. The water-insoluble product (H<sub>2</sub>O-I) contained 13 per cent nitrogen and 3 per cent ash and represented about 80 per cent of the defatted tissue. The water extracts, combined and lyophilized, resulted in a water-soluble product (H<sub>2</sub>O-S); a nonhygroscopic, easily powdered, yellow solid containing 11 per cent nitrogen and 22 per cent ash.

These tumor fractions, H<sub>2</sub>O-I and H<sub>2</sub>O-S,

and certain other known compounds tested (sodium chloride, potassium chloride, calcium acetate, calcium citrate, magnesium citrate, magnesium carbonate, ammonium citrate, sodium citrate and sodium acetate) were incorporated into diets or fed as supplements to a 20 per cent casein diet, C-20. Both the C-20 diet and a diet (T-24) containing 24 per cent lyophilized Walker 256 tumor tissue were used as standards for comparison. All diets were isocaloric and isonitrogenous, adjustments for differences in nitrogen being made by varying the quantity of starch in the diets.

The experimental diets were fed to Sprague-Dawley rats bearing the transplanted Walker 256 tumor, beginning 15 days after tumor transplantation. Two types of feeding procedures were used. In one procedure, all animals were fed the C-20 diet until they failed to gain weight, at which time they were given an experimental diet or supplement and the effect on changes on weight and food intake observed. In the second procedure, comparisons were made among groups of six to ten rats ingesting the experimental and control diets throughout the course of the experiment.

Food intakes and weights were measured daily. The animals were killed at the end of all experiments, their tumors and adrenals removed and weighed. In one experiment, carcasses (minus adrenals, livers and intestinal contents) and tumors were weighed, lyophilized and defatted with 3:1 alcohol-

ether followed by 1:1 chloroform methanol. The residues, washed with alcohol-ether, were lyophilized, the weight of the residue representing the fat-free dry weight. Fat content was calculated by difference.

Utilizing the first type of feeding procedure, the authors tested the water-insoluble fraction ( $H_2O$ -I) as a nitrogen source at the 22 per cent level ( $H_2O$ -I-22) and the water-soluble fraction ( $H_2O$ -S), added to the casein control diet C-20, at a concentration (4.5 per cent) comparable to its level in the whole tumor tissue diet, T-24. Diet  $H_2O$ -I-22, on replacing C-20 on the fourteenth day, failed to produce an increase in growth or food intake. However, addition of  $H_2O$ -S to diet C-20 produced an increase in weight parallel to that of animals fed diet T-24 throughout the experiment.

These findings suggested to the authors that a growth-stimulating factor resided in the water-soluble fraction and led them to purify further  $H_2O$ -S by putting an alcohol extract of that fraction through a charcoal column. The forerun yielded a product containing only 3 per cent nitrogen and having a high inorganic salt content (13 per cent sodium and 13 per cent potassium). The inorganic fraction, fed as a supplement to diet C-20, produced dramatic overnight weight gains in a rat, mostly due to an increased water intake.

Subsequent experiments, in which a variety of inorganic salts (see above) were fed as supplements (0.1 to 0.2 g. per day) to animals that had attained a plateau on the C-20 diet, suggested that the sodium ion was the specific cation responsible for the stimulating effect.

Utilizing the second type of feeding procedure, the authors compared the effects of additional dietary sodium chloride on the total body weight of tumor-bearing rats and on the weight change and composition of carcass and tumor at two levels (0.9 and 1.8 per cent of the diet) with the effects of diets C-20 and T-24. All diets contained

initially a salt mixture (R. B. Hubbell, L. B. Mendel and A. J. Wakeman, *J. Nutrition* 14, 273 (1937)) supplying 0.3 per cent sodium chloride. Diet C-20 plus 0.9 per cent sodium chloride was equivalent in sodium content to diet T-24. When the four diets were fed ad libitum to rats bearing 15-day-old tumors, the two salt-containing diets were as effective as T-24 for the greater part of the experiment, *i.e.*, for 19 to 23 days. Increasing the sodium chloride content twofold had no increased effect. The total body weight, including tumor, and the weight of the tumor were greatest for the group on the T-24 diet, although animals receiving the high-salt diets had the greatest food intake.

In a separate experiment, tumor-bearing rats on the C-20 plus 1.8 per cent sodium chloride diet were pair-fed to a group ingesting T-24. Their gains in weight were less than those of rats on T-24 or C-20 fed ad libitum, indicating, in the author's opinion, that the growth rates of animals ingesting salt diets ad libitum (which matched the rates of animals ingesting T-24) could be attributed to increased food intake. These experiments led the authors to conclude that sodium chloride cannot duplicate tumor effects completely.

The adrenal weights of animals bearing large tumors and ingesting C-20 were greater by a factor of three than those of normal control animals. However, adrenal weights of animals ingesting T-24 or the high-salt diets were slightly less than twice the weights of the normal controls. The *zonae glomerulosae* in adrenals of animals ingesting the high-salt diets were appreciably narrower and had lower lipid contents, as judged from Oil Red O stain. The authors interpret these adrenal findings as indicating that salt prevents an adrenal hypertrophy in tumor-bearing animals, which, they believe, have an increased demand for salt.

Carcass and tumor composition of Walker 256 tumor-bearing rats ingesting the C-20, T-24 and C-20 plus 1.8 per cent sodium

chloride diets ad libitum were measured as described above in one experiment. On all three diets, the rats lost carcass weight as the tumors increased in size. Inspection of the carcass analysis data reveals that, on the C-20 diet, about 60 per cent of the carcass weight loss was lost as water, 17 per cent as fat-free dry weight, and 24 per cent as fat. On the T-24 diet, about 49 per cent of carcass weight loss was lost as water, 23 per cent as fat-free dry weight, and 28 per cent as fat. On the C-20 plus 1.8 per cent sodium chloride diet, 53 per cent of carcass weight loss was lost as water, 19 per cent as fat-free dry weight, and 28 per cent as fat. Figures for water as a percentage of fat-free wet weight were slightly higher for the T-24 and C-20 plus 1.8 per cent sodium chloride diets (76.2 and 74.7 per cent, respectively) than for the C-20 diet (73.0 per cent), but only slightly different from the control percentage (74.4). There was no apparent influence of diet on the water content of tumor. The authors believe these data are additional evidence for the presence of a disturbance in water and electrolyte metabolism in these tumor-bearing rats.

As evidence for a possible factor in the water-insoluble fraction ( $H_2O-I$ ), the authors cite data obtained under conditions of prolonged  $H_2O-I$  feeding. When three groups of rats bearing 15-day tumors were fed on C-20, T-24 and  $H_2O-I-22$ , respectively, for 25 days, the animals on C-20 reached a weight plateau, those on T-24 continued to grow and those on  $H_2O-I-22$  showed first a delay in growth but then a growth rate almost equal to that of animals on the T-24

diet. The delay was associated with a 15 to 25 per cent decrease in food intake. Rats fed  $H_2O-I-22$  grew tumors equal to or larger than those fed T-24, and both groups had larger tumors than those fed C-20. However, carcass weight loss was greatest in animals fed  $H_2O-I-22$ . The possibility that the effect of  $H_2O-I-22$  on tumor growth might be due to its content of sodium ion was ruled out by the low ash content (3 per cent) of the  $H_2O-I$  fraction. When the experiment was repeated with an additional group of rats ingesting  $H_2O-I-22$  into which 0.9 per cent sodium chloride had been incorporated, the group receiving  $H_2O-I-22$  plus added salt grew the largest tumors but showed less carcass weight loss than any group except that ingesting T-24.

This work demonstrates the necessity of isolating and testing biologically the individual components of so heterogeneous a tissue as Walker carcinosarcoma 256 if sense is to be made of the appetite and growth-stimulating effects of tumor-containing diets. The well-known effects of sodium ion on water metabolism and the failure of sodium ion supplementation of the C-20 diet to duplicate the effects of the T-24 diet over extended periods suggest more strongly than the authors indicate that the significant developments in this problem lie ahead. Experiments designed to eliminate the distractions produced by the sodium ion are indicated and reports of such studies, following a promised communication on electrolyte balance studies, may be anticipated.

### LYSINE AS A CATION IN POTASSIUM DEFICIENCY

*Lysine appears to function as a cation contributing to the maintenance of ion balance in the muscle cells of potassium-deficient animals.*

The loss of potassium from skeletal muscle of rats fed a diet deficient in potassium is accompanied by an increase in intracellular sodium equivalent to one-half to two-thirds

the amount of potassium lost (H. C. Miller and D. C. Darrow, *Am. J. Physiol.* **130**, 747 (1940); E. Muntwyler and G. E. Griffin, *J. Biol. Chem.* **193**, 563 (1951)). Thus, muscle

of potassium-deficient animals is relatively deficient in alkali metal cations.

The following observations have suggested that the apparent deficit is compensated by an increase in intracellular hydrogen ions. (1) During recovery from potassium deficiency, the alkali cation deficit of muscle is corrected and rats lose hydrogen ions in an amount roughly equal to the deficit (R. E. Cooke *et al.*, *J. Clin. Invest.* **31**, 798 (1952)). (2) L. I. Gardner *et al.* (*J. Gen. Physiol.* **36**, 153 (1952)) found the intracellular pH of muscle from potassium-deficient animals (calculated from carbonic acid determinations) to be lower than that of muscle from control animals. Finally (3), J. Orloff *et al.* (*J. Clin. Invest.* **32**, 538 (1953)) found that plasma pH decreased following injection of potassium chloride into nephrectomized potassium-deficient rats. No plasma acidification occurred when sodium chloride was injected.

An hypothesis that cations other than hydrogen may increase in the cells during potassium deficiency has been suggested by R. E. Eckel, J. E. C. Norris and C. E. Pope II (*Am. J. Physiol.* **193**, 644, 653 (1958)). This possibility, that a portion of the potassium deficit might be made up by an increase in the concentration of cations not usually determined, was in part suggested by the work of H. N. Christensen *et al.* (*J. Biol. Chem.* **198**, 17 (1952)), who found that 2,4-diaminobutyric acid competes with potassium for entrance into a type of tumor cell.

In their studies to test this hypothesis Eckel *et al.* (*loc. cit.*) produced potassium deficiency in rats either by simple dietary deficiency or by steroid administration; or, for very rapid production, by protein depletion followed by the feeding of a high-protein, potassium-free diet. Male rats initially weighing 100 to 140 g. were kept on the depletion regimen from 13 to 29 days. Sodium and potassium concentrations of muscle, plasma and urine were determined by flame photometry, chloride by electro-metric titration, and lysine was measured

quantitatively by microbiological assay. Total amino acids were measured by paper electrophoresis and colorimetric reaction with copper sulfate.

A cation which was found in increased amounts in muscles from potassium-deficient rats was identified as L-lysine by four methods. (1) Using paper electrophoresis, the behavior of the cation was compared at two pH values and in three different solvents with the behavior of lysine, arginine and ornithine. (2) The absorption spectrum of the reaction product of the cation with ninhydrin was determined. (3) The theoretical yield of carbon dioxide and appearance of cadaverine were obtained after the action of lysine decarboxylase. Finally (4), the growth of *Leuconostoc mesenteroides* P-60 on a lysine-free medium supplemented with the cation was measured.

Rats treated with desoxycorticosterone acetate grew normally. However, they developed hypokalemic alkalosis and lowered plasma and muscle potassium concentrations just as did rats fed the potassium-deficient diets, indicating that these changes are not responsible for the poor growth and failure of protein synthesis in the potassium-deficient rats.

The most interesting observation from the first study was the four to tenfold increase in lysine and in total basic amino acids in the muscle of potassium-deficient rats, regardless of the cause of the potassium deficiency. Plasma lysine was increased in dietary potassium deficiency but was not consistently changed by the other two treatments. In rats kept on a potassium-deficient diet for two weeks, increase in basic amino acids accounted for the whole alkali-metal cation deficit. However, in rats kept on potassium-deficient diets for four weeks, the basic amino acids accounted for only one-third of the deficit. There was no increase in acidic amino acids nor any other change in the muscle amino acids of the deficient rats. Although potassium-deficient rats, repleted by potassium chloride injections,

had slightly decreased muscle basic amino acids when compared to sodium chloride-injected rats, there was no increase in excretion of lysine or lysine metabolites.

The second study reported by Eckel *et al.* (*loc. cit.*) was designed to show whether lysine and potassium compete for entrance into the cell and whether lysine affects the renal excretion of potassium. Muscle electrolytes and amino acids of rats fed the control or the potassium-deficient diet, supplemented with 10 per cent of L-lysine as the hydrochloride, were determined. The effects on muscle constituents and urinary potassium of supplementing the deficient diet with lysine hydrochloride or with ammonium chloride were also determined. In both series, the group with the lower food consumption determined the amount of food fed to the other group.

The concentration of lysine in the muscles of control and potassium-deficient rats receiving a lysine supplement increased well above values for the comparable unsupplemented groups. Lysine supplementation did not decrease muscle potassium nor increase potassium excretion above that caused by ammonium chloride supplementation. Thus these observations demonstrate that accumulation of basic amino acids does not, in itself, cause loss of potassium from muscle, and indicate that lysine and potassium do not compete for entrance into the cell or for reabsorption or excretion by the kidney.

The observations that basic amino acids accumulate in the absence of potassium depletion, and that the concentrations of both potassium and basic amino acids in muscle decrease as the potassium depletion period is extended from 13 to 29 days, make it difficult to estimate the significance of the accumulation of basic amino acids in muscle. However, the observation that there is a decrease in muscle basic amino acids during potassium repletion (as reported in their first study) supports their hypothesis explaining the increased hydrogen ion content of urine during potassium repletion.

According to their scheme, during repletion, lysine leaves the muscle cells accompanied by hydrogen ions and this results in the urinary excretion of 1 mole of hydrogen ions per mole of lysine extruded from the cell.

These studies by Eckel *et al.* indicate that changes which would cause intracellular acidosis, *i.e.*, deficiency of cations, weak bases or excess of anions, do not occur in the muscle of potassium-deficient rats. However, previous workers (L. I. Gardner *et al.*, *J. Gen. Physiol.* **36**, 153 (1952)) have reported a decreased intracellular pH in potassium deficiency. Thus, in order to study the possibility of intracellular acidosis, Eckel, A. W. Botschner and D. H. Wood (*Am. J. Physiol.* **196**, 811 (1959)) studied the buffering capacity, ionic equivalence by paper electrophoresis, organic acid content, and the pH of muscle by carbon dioxide determination and by direct measurement. Throughout the study, careful attention was given to possible methodological sources of error.

No differences between control and potassium-deficient muscle were revealed by these techniques. Different absolute values for pH (pH 7.1 *versus* 6.9) were obtained by the two methods, but differences between control and deficient muscles did not exceed 0.06 to 0.08 pH units. The pH and buffering capacity of potassium-deficient muscle was about the same as that of normal muscle.

These studies have demonstrated that, early in potassium deficiency, increased amounts of lysine are found intracellularly; that there is no evidence for an increase in intracellular hydrogen ion concentration during this time; and that lysine does not compete with potassium for entry into the cell. When rats remain on potassium-deficient diets for as long as four weeks, muscle lysine decreases. It is, therefore, possible that the characteristics of muscle from animals suffering a prolonged deficiency of potassium may differ from those described here, which were observed rela-

tively early during the development of potassium deficiency. The main point of interest for nutritionists is the possibility

that lysine may function as a cation contributing to the maintenance of ion balance in muscle cells.

## SCURVY AND BLOOD COAGULATION

*The vascular abnormalities resulting from a deficiency of ascorbic acid include a change in capillary permeability, anemia, a reduction in platelets, an increase in fibrinogen and a reduction in the levels of some of the plasma components required for blood coagulation.*

Petechial hemorrhage is a prominent symptom of ascorbic acid deficiency. It is generally accepted that the primary disturbance producing the petechiae is a change in the permeability of the capillaries. However, over the years there have been reports that there are additional changes in scorbutic individuals and animals involving the coagulability of the blood and its content of cellular components.

The most commonly recognized blood change in scurvy is the anemia (*Nutrition Reviews* 3, 178 (1945)). The anemia seen in adult scurvy responds promptly to ascorbic acid supplementation. It is still impossible, however, to determine the extent to which ascorbic acid increases the hemoglobin level by an action on the hemopoietic system or by facilitating the absorption of iron (*Ibid.* 13, 165 (1955); 16, 113 (1958)).

There is less agreement as to the changes in blood coagulation associated with a deficiency of ascorbic acid. Although considerable work has been done on the coagulation defect in this deficiency state, there is considerable divergence of opinion as to the magnitude and cause of the alteration.

R. J. Salmon and C. D. May (*J. Nutrition* 46, 515 (1952)) found evidence in monkeys "that ascorbic acid plays a role in the production of plasma fibrinogen. . . ." In 1943, W. R. Sullivan, E. O. Gangstad and K. P. Link (*J. Biol. Chem.* 151, 477 (1943)) observed a prolongation in the prothrombin time of the plasma of guinea pigs from 28 seconds during the control period to 40

seconds when they developed scurvy. The prothrombin time increased to as much as 200 seconds after a 5 mg. dose of 3,3'-methylenebis-(4-hydroxycoumarin) and stayed that way for as much as two weeks. Normal guinea pigs receiving 10 mg. of the anticoagulant showed a prolongation of the clotting time to about 80 seconds with a return to normal in 48 hours (R. S. Overman *et al.*, *J. Biol. Chem.* 142, 941 (1942)). The ability to restore normal prothrombin levels after the administration of an anticoagulant is markedly reduced.

A. K. Presnell (*J. Nutrition* 8, 69 (1934)) observed that the coagulation time of blood taken from an ear vein increased within three weeks after the guinea pigs were put on a scorbutogenic diet to 4.9 minutes compared to 2.7 for the controls.

Recently, P. Barkhan and A. N. Howard (*Brit. J. Nutrition* 13, 389 (1959)) studied the changes in blood constituents during the development of scurvy in guinea pigs. They fed 250 g. guinea pigs the purified diet developed by M. E. Reid and G. M. Briggs (*J. Nutrition* 51, 341 (1953)) until the animals attained a weight of 300 g. Then 13 of them were put on the diet from which the ascorbic acid had been removed. These animals continued to grow normally for ten or 12 days and then lost weight, with death occurring after 22 to 28 days. At the time of death, the guinea pigs weighed 190 g.

After being on the scorbutogenic diet for ten days, the five guinea pigs tested had a platelet count of  $900 \pm 177 \times 10^{-3}$  per



cubic millimeter compared to  $491 \pm 39$  in the controls. On the tenth day, the prothrombin time as measured by the one-stage technique was  $28.2 \pm 0.4$  seconds compared to  $26.1 \pm 0.6$  in the controls. By the nineteenth to the twenty-fourth day of the scorbutic regimen, the animals showed no further increase in platelets but a prolongation of the prothrombin time to  $32.7 \pm 1.5$  seconds.

To determine the specific factors in blood which were responsible for the prolonged prothrombin time, Barkhan and Howard (*loc. cit.*) carried out a number of tests which provided presumptive evidence for deficiencies, especially of the thromboplastic components of blood and the plasma accelerators or co-factors essential for the initiation of thromboplastic activity.

At this point, it becomes necessary to digress for a moment in order to indicate that one of the more widely accepted theories of blood coagulation (R. Biggs and R. G. MacFarlane, *Human Blood Coagulation and its Disorders*, second edition. Blackwell, Oxford (1957)) is that thromboplastin is formed by the interaction of a number of blood components. Those that are most clearly defined are: (1) platelets, (2) anti-hemophilic globulin (AHG), and (3) the plasma thromboplastic component (PTC). One of the latter two components is missing from the blood of true hemophiliacs, and the two types of hemophilia are classified on the basis of which of these blood coagulation components is lacking. The three components react with calcium and other less clearly defined components to form thromboplastin. The less clearly defined components have been recognized from studies of various congenital hemorrhagic diseases; these include the plasma thromboplastic antecedent factor (PTA), the Stuart factor, Factor I and others.

The conversion of prothrombin to thrombin occurs under the influence of thromboplastin and is apparently accelerated by at least two other well-defined substances,

Factor V and Factor VII. The blood level of the latter (Factor VII) is reduced together with that of prothrombin by dicoumarin-like compounds.

The studies of Barkhan and Howard indicated that there may be low blood levels of Factor VII as well as plasma and serum thromboplastic factors (PTA and PTC) in scurvy. Their conclusions are open to some question since there is no way of knowing whether the presumptive tests for deficiencies of coagulation factors found in human blood are applicable to animal blood. Some factors, particularly the accelerator, Factor VII, show marked species differences in properties and blood concentrations. Furthermore, the abnormalities observed by Barkhan and Howard were quite variable (they were seen in about 50 per cent of their guinea pigs) and involved the less clearly defined thromboplastic components of the coagulation scheme.

It is interesting that, regardless of the type of defect, it could not be corrected by an injection of ascorbic acid a few hours prior to withdrawing the blood sample.

The blood from the normal and scorbutic animals retracted equally well with the clots maintaining their integrity at  $37^{\circ}\text{C}$  for at least five hours. This indicated that there were no gross abnormalities in clot retraction and lysis in the blood of the scorbutic guinea pig. In addition, blood from the scorbutic and control guinea pigs gave similar results in the assays for Factor V.

Scurvy appears to influence the blood-vascular system in a number of ways. The abnormality in vascular permeability and the presence of anemia are well documented. There appears to be an abnormality of blood coagulation, an increase in blood fibrinogen and possibly also in platelet levels. Variable changes probably occur in some of the blood components involved in the coagulation process, but these changes are likely to be of no physiological significance.

Approaches have been made to identify the specific factors responsible for the blood

coagulation abnormality. The work done so far suggests that the thromboplastic factors and certain co-factors or accelerators of thromboplastic activity are involved. More work is required before a final decision can be made as to factors that are deficient.

Much of the older work with scorbutic animals is complicated by the presence of concomitant nutritional deficiencies. Today

it is possible to produce an uncomplicated ascorbic acid deficiency in both guinea pigs and monkeys, and techniques have been perfected for the more precise assay of a variety of the constituents that are involved in blood coagulation. Here is a field awaiting the active collaboration of competent hematologists and nutritionists.

### SATURATION OF DIETARY FATS AND LIVER AND SERUM LIPIDS

*Thirteen edible fats were fed to rats during a post-weaning period of seven weeks. Liver and serum cholesterol values were determined as well as the distribution of unsaturated fatty acids in cholesterol esters.*

It has been demonstrated that diets containing large amounts of unsaturated fatty acids promote the reduction of serum cholesterol levels (L. W. Kinsell *et al.*, *J. Clin. Nutrition* 1, 224 (1953)). This action may be the result of essential fatty acids facilitating the transport or catabolism of cholesterol, thus preventing its deposition in arterial walls.

The action of dietary linoleate may be catalytic and require only trace amounts. It could, on the other hand, require relatively large quantities so that considerable amounts of cholesterol could be esterified. The fact that therapeutic diets rich in unsaturated fat are high in calories may be a disadvantage. Accordingly, experiments which help to clarify the role of unsaturated fatty acids in cholesterol metabolism are valuable. Although much significance is attached to the level of circulating cholesterol, it does not necessarily indicate the amount of tissue cholesterol or its ease of mobilization.

Experiments have been reported by R. Okey *et al.* (*Metabolism* 8, 241 (1959)) in which the effect of unsaturation of dietary fats on liver and serum lipids has been studied. These experiments were designed to determine the extent to which the fatty acids of liver cholesterol esters were altered by feeding fats of differing compositions.

Each of 13 fats were added so as to make up 10 per cent of an adequate fat-free basal diet. Each of these diets was fed to a group of ten male and 10 female rats, while a second group of 20 rats received a 1 per cent cholesterol supplement added to each diet. The feeding began at weaning and lasted seven weeks. Tissue samples were extracted to determine total lipids and cholesterol, and cholesterol esters were isolated using silicic acid columns. After saponification and extraction of cholesterol, the fatty acids were acidified and extracted. Spectrophotometric analysis was used to determine the percentages of polyunsaturated fatty acids. As a further check, silicic acid paper chromatography was used. This gave information about the monoenoic acid content.

Assorted oils were used, including the following listed in order of decreasing iodine number: menhaden, safflower, corn, soy, cottonseed straight and cottonseed hardened, peanut, olive, lard, shortening, butter oil, butterfat and coconut. The per cent content of monoene, diene and polyene fatty acids was determined.

Weight gains of males averaged between 225 and 272 g. without cholesterol, and between 195 and 244 g. with cholesterol. Females without the cholesterol supplement averaged between 141 and 169 g. gained.

With cholesterol, the females averaged between 137 and 177 g. The efficiency, or gain per gram of diet eaten, was similar in all males, ranging from 0.32 to 0.40 g. In the females, the lowest gain per gram eaten was 0.25 g. and the highest was 0.34. There was no apparent relationship between diet content of unsaturated fatty acids and the amount of weight gain shown.

Liver weights ranged between 8.3 and 14.3 g. in males and between 5.2 to 8.2 g. in females.

Liver free cholesterol averages fell to between 0.16 and 0.26 g. per cent in both males and females without cholesterol. Values for males receiving cholesterol extended from 0.22 to 0.39 g. and, for females, from 0.25 to 0.41.

Serum cholesterol levels averaged from 53 to 84 mg. per cent in males and from 50 to 86 mg. per cent in females when no cholesterol was fed. When males were fed cholesterol, they averaged from 55 to 96 mg. per cent and, in females fed cholesterol, from 68 to 219 mg. per cent.

In discussing their experiments, Okey *et al.* point out that their diets did not contain enough fat to induce fatty livers. Their purpose was to measure the result of alteration in composition of dietary fat on the retention of food cholesterol. One trend observed in the young males was that liver cholesterol values decreased as iodine numbers decreased, but that serum cholesterol levels remained relatively constant. This same finding was reported by C. H. Best, C. C. Lucas, J. M. Patterson and J. G. Ridout (*Canad. J. Biochem. Physiol.* **36**, 613 (1958)), although their experiments were not strictly comparable.

As the mean chain length of dietary fatty acids increased, the liver lipids and cholesterol levels tended to increase. The cholesterol liver storage observed in rats fed hardened cottonseed oil suggested that long-chain saturated fatty acids may have been influential. Cholesterol-fed females fed safflower seed oil, cottonseed oil, olive

oil, lard, shortening and coconut oil had high serum cholesterol levels. Two possible causes were proposed by the authors. One is the solubility of cholesterol esters in the dietary fatty acids, and another, the possibility that some substance in the oils inhibited hepatic destruction of estrogen, thus allowing more effect on serum cholesterol. The males fed cholesterol had a lower level of circulating cholesterol and higher liver lipids than the females, with the exception that liver cholesterol was higher in the females when the rats were fed menhaden, safflower, corn and olive oil.

Circulating cholesterol is utilized in intermediary metabolism and restored from the liver reservoir, and the rat appears to be unharmed while efficiently storing large quantities of cholesterol in the liver. If this storage is pathological, then the data on growth and liver and serum cholesterol do not suggest that dietary essential fatty acids or linoleate are able to control the accumulation.

Analysis for unsaturated fatty acids of liver cholesterol esters appear to reflect the pattern of these compounds determined in the diet oils.

Oleic acid appeared to be a prominent constituent of liver cholesterol esters, even when dietary fat was not rich in oleate. Several questions are suggested in studying the data found in these experiments. Is the liver cholesterol oleate easily taken up by the blood? Does the fact that male animals have more liver cholesterol oleate than females have any bearing on the high serum cholesterol levels of females? What is the significance of the finding that females have a high serum cholesterol when fed coconut oil, which is essentially without linoleate to assist in cholesterol transport? Why does arachidonate, a tetraenoic acid, appear as a constituent of cholesterol esters in animals receiving a diet low in linoleate if linoleate is the only precursor of arachidonate?

The answers to these questions, as well as more detailed knowledge of the fatty acid spectrum of lipids associated with pathological states including arteriosclerosis, require further study. If one assumes, as is usually justifiable, that the serum cholesterol concentration gives the best index of whether or not conditions are favorable for the occurrence of atherosclerosis, then some of the serum cholesterol values obtained in these experiments are very difficult to inter-

pret. There is already considerable experimental evidence suggesting that some of the fats which led to the relatively high serum cholesterol values in the females in this experiment are highly protective against fatty arterial disease in the rat.

It should be pointed out, however, that the results of this type of experiment might be quite different with a higher or lower protein intake or with older animals or with a different length of experimental feeding.

### EFFECTS OF DIETARY FAT, INOSITOL, VITAMIN B<sub>12</sub> AND GLUCOSE-CYCLO-ACETOACETATE ON COAGULATION

*Essential fatty acids, inositol, vitamin B<sub>12</sub> and glucose-cyclo-acetoacetate partially inhibited an increase in blood platelets, coagulability and various lipids of some tissues and sera of rabbits fed 20 per cent saturated peanut oil.*

A controversy related to cardiovascular investigation concerns the possible effects of dietary fat on coagulation and thrombolysis (*Nutrition Reviews* 17, 218, 263 (1959)). Meals high in fat, particularly if the fat is saturated, have been reported to produce shortening of clotting time. This has been observed in dogs and humans after ingestion of cream (G. G. Duncan and J. M. Waldron, *Trans. Assoc. Am. Physicians* 62, 179 (1949)), in humans fed animal fat (H. W. Fullerton, W. J. A. Davie and G. Anastasopoulos, *Brit. Med. J.* 2, 250 (1953)), in man fed meals of saturated or unsaturated fat (A. Keys, R. Buzina, F. Grande and J. T. Anderson, *Circulation* 15, 274 (1957)), and in man after ingestion of butter (N. F. MacLagan and J. D. Billimoria, *Lancet* II, 235 (1956)). Reduction of platelets and clotting time was observed in rabbits fed cream by R. L. Swank and C. F. Cullen (*Proc. Soc. Exp. Biol. Med.* 82, 381 (1953)). In addition, both *in vitro* and *in vivo* in animals and *in vitro* in man, saturated fats inhibit fibrinolysis activated by streptokinase (W. A. Thomas and R. F. Scott, *Ibid.* 96, 24 (1957)).

Similar findings have been reported by

other groups (Fullerton, *Brit. Med. J.* 1, 663 (1955); H. B. W. Grieg, *Ibid* 2, 708 (1957); E. Sohar, M. C. Rosenthal and D. Adlersberg, *Am. J. Clin. Path.* 27, 503 (1957)). Some of these results may be relevant to the cholesterologenic and atherogenic effects of saturated fats under certain dietary conditions in animals (*Nutrition Reviews* 18, 23 (1960)). It has also been shown that saturated fats incorporated into rather unphysiologic food mixtures favor a high incidence of myocardial infarcts in rats (Thomas, W. S. Hartroft and R. M. O'Neal, *J. Nutrition* 69, 325 (1960)).

However, there are other reports in which such effects of dietary fat (animal or vegetable) have not been obtained respecting either coagulation or fibrinolysis (G. H. Hall, *Brit. Med. J.* 2, 207 (1956); C. Merskey and H. L. Nossell, *Lancet* I, 806 (1957); S. I. Nitzberg *et al.*, *Circulation* 19, 676 (1959)). Nitzberg *et al.*, however, did find a shortening of Stypven time in hyperlipemic man and prolongation of profibrinolytic activity. A comprehensive review of dietary fats and blood coagulation recently published emphasizes much of the controversy (S. A. Hashim and R. E. Clancy, *New Engl. J. Med.* 259, 1115 (1958)).

A report by M. C. Nath and A. Saikia (*J. Nutrition* 69, 403 (1959)) may help elucidate aspects of this controversy. Their results lend support to the hypothesis that saturated fats fed to rabbits both enhance experimental atherosclerosis and favor coagulation (shortening bleeding time, coagulation time, prothrombin time). Their experiments differ somewhat from previously published ones in that they controlled the levels of essential fatty acids, inositol, vitamin B<sub>12</sub> and glucose-cyclo-acetoacetate in their food mixtures (*Indian J. Med. Res.* 47, 73 (1959)).

The controls received native peanut oil (6 per cent), whereas the experimental rabbits received 20 per cent hydrogenated peanut oil. The remainder of the diet corresponded to semi-synthetic mixtures used in this country (casein 20 per cent, sucrose 30 per cent, wheat flour 39 per cent, salt mixture 5 per cent, and vitamin mixture 3 per cent, by weight). The experimental diets with the higher fat content were made isocaloric with the control at the expense of the carbohydrate. Additional experimental groups received 1 per cent linoleic acid, linolenic acid or inositol added to the saturated peanut oil. Other groups received, in addition to saturated fat, daily injections of either 15 micrograms of vitamin B<sub>12</sub> per kg. body weight or 300 mg. per kg. body weight of hydrolyzed glucose-cyclo-acetoacetate.

After 12 weeks on the above regimens, the bleeding time, coagulation time, prothrombin time, blood platelet counts and hemoglobin contents of the blood were determined in the fasting animals. The animals were then sacrificed and total and esterified cholesterol levels as well as lipid phosphorus levels of the serum measured. Hepatic and cardiac lipids (total, free cholesterol, ester cholesterol, phospholipid) were estimated biochemically.

The authors reported statistically significant effects of feeding the high levels of dietary saturated fat on the bleeding time, coagulation time, and prothrombin time, all

of which were shortened. There were also small but significant increases in the number of blood platelets and levels of total lipids and ester cholesterol of blood, liver and heart. The supplements of essential fatty acids (linoleic and linolenic), inositol, vitamin B<sub>12</sub> and hydrolyzed glucose-cyclo-acetoacetate counteracted partially (but not completely) the shortening of the coagulation factors, although the effect of linoleic acid here was of questionable significance. The authors suggest that the increase in the number of blood platelets of the rabbits fed the saturated fat (which was prevented somewhat by the supplements of essential fatty acids, inositol, vitamin B<sub>12</sub> or glucose-cyclo-acetoacetate) might be responsible for the increased coagulability of blood.

Although the magnitude of some of the differences reported was not great, the differences are of sufficient interest to warrant further investigation. These experiments emphasize the complexity of the relationships between dietary fat and thrombosis and indicate that many factors may be involved, of which the ones investigated may represent only a few. Moreover, these experiments indicate the difficulty of comparing results from several laboratories where these factors may vary.

Before extensive surveys of coagulation in man with respect to dietary fats are undertaken on any large scale, further animal experimentation is indicated in the light of this report by Nath and Saikia. One urgent question is the relationship between the cholesterogenic effect on serum by saturated fats and their effect on thrombosis and fibrinolysis. It is important to know whether these two effects of dietary fat are inter-related or merely coincidental. As far as this reviewer is aware a direct effect of cholesterol on coagulation or fibrinolysis has not been demonstrated. This question would appear to be of paramount importance, and the report of Nath and Saika emphasizes this need.



## GLYCERIDE SYNTHESIS DURING FATTY ACID ABSORPTION

*Triglyceride formation has been demonstrated during intestinal absorption of fatty acids in vitro. Homogenates of intestinal mucosa failed to activate free glycerol, indicating it is probably not a triglyceride precursor.*

For over half a century it has been known that fatty acids ingested as simple esters appear in triglycerides in the thoracic duct chyle (T. P. Hilditch, *The Chemical Constitution of Natural Fats*, second edition, p. 368. Wiley and Sons, New York (1947)). Now, J. M. Johnston (*J. Biol. Chem.* **234**, 1065 (1959)) has adapted an *in vitro* technique for the study of esterification of fatty acids during their passage through the intestinal wall. Since glycerol fed with fatty acids is not found in chyle triglycerides (R. Reiser *et al.*, *Ibid.* **194**, 131 (1952)), the question of the source of the glycerol for triglyceride synthesis in the intestinal mucosa has been the subject of an *in vitro* study by G. C. Buell and Reiser (*Ibid.* **234**, 217 (1959)).

In his study of fatty acid absorption *in vitro*, Johnston (*loc. cit.*) used everted sacs of small intestine from the golden hamster, a preparation that has been used so successfully for studying amino acid and carbohydrate absorption. The mucosal side of the intestinal segment was bathed in 25 ml. of a solution containing C<sup>14</sup>-labeled palmitic acid-albumin complex and glucose. The serosal side was bathed in a buffered solution containing only glucose. The experimental procedure permitted the removal of samples at intervals during the incubation (T. H. Wilson, *J. Applied Physiol.* **9**, 137 (1956)).

The sacs were incubated for 2.5 hours at 37°C and aliquots of the mucosal and serosal solutions removed at half-hour intervals. Lipids were extracted from each aliquot of the serosal and mucosal solutions and from the intestine itself at the end of the experiment. Free and esterified fatty acids were separated and the radioactivity of each fraction was determined. Finally, the esterified fatty acids from the serosal solution

were dissolved in acetone and subjected to chromatography on a silicic acid column to ensure that esters other than triglycerides were removed.

From 2.5 to 7.5 per cent of the radioactivity was transferred from the mucosal to the serosal side of the everted intestine and 11 per cent was found in the intestinal wall. Of the activity remaining on the mucosal side, 84 per cent was recovered as free fatty acids and 16 per cent as esterified fatty acids; the respective figures for the intestinal wall were 20 and 80 per cent, and for the serosal solution 13 and 87 per cent. Thus most of the fatty acids passing through the intestinal wall were esterified. The chromatographic separation of the esters indicated that these were almost exclusively di- and triglycerides with by far the greatest portion of the radioactivity being in the triglycerides.

The investigation by Buell and Reiser (*loc. cit.*) was concerned with the precursors of glyceride-glycerol in the intestinal mucosa. They used pig intestine as their starting material. The mucosa was removed and homogenized in 0.6 per cent sodium chloride. The homogenate was then centrifuged at low speed to remove cell debris and the supernatant solution was used as a source of the enzymes required for triglyceride synthesis. In some instances the preparation was centrifuged a second time to remove mitochondria as well.

The incubation mixture contained, besides the intestinal mucosa homogenate, palmitic acid, reduced diphosphopyridine nucleotide, coenzyme A, glutathione, adenosine diphosphate, magnesium chloride, phosphate buffer (pH 7.0), aldolase, and uniformly C<sup>14</sup>-labeled fructose diphosphate. The reaction



mixture was incubated under anaerobic conditions for two hours at 40°C, after which the enzyme was inactivated by boiling. The lipids were then removed by solvent extraction, dried, and dissolved in ether. Phospholipids, free fatty acids and monoglycerides were removed and the remaining lipid was identified as triglyceride by a chromatographic technique. The triglyceride was then dissolved in Skellysolve B, plated, and the radioactivity determined.

From 20 to 35 per cent of the radioactivity of the labeled fructose 1,6-diphosphate was incorporated into the isolated triglycerides, indicating clearly that this compound serves as a precursor of glyceride-glycerol. In order to determine whether free glycerol or other glycerol precursors were incorporated into glycerides by the intestinal mucosa, unlabeled precursors were added to the complete incubation mixture. If an unlabeled substance were a precursor of glyceride-glycerol, less of the radioactivity from fruc-

tose diphosphate would be incorporated into the triglycerides.

The addition of either dihydroxyacetone phosphate or L-alpha-glycerophosphate to the incubation mixture reduced the amount of radioactivity incorporated by 30 to 65 per cent. However, when free glycerol was added, no significant reduction in the radioactivity of the isolated triglycerides was observed. The authors conclude, therefore, that intestinal mucosa, in contrast to liver (E. P. Kennedy, *Ann. Rev. Biochem.* **26**, 123 (1957)), does not contain glycerol kinase and that L-alpha-glycerophosphate is the immediate precursor of glyceride-glycerol. Thus it is not surprising that glycerol fed with free fatty acids does not appear in lymph triglycerides.

The results of these two *in vitro* studies are in accord with previous observations on intact animals and add to the evidence that triglyceride synthesis in the intestinal wall is an important step in fatty acid absorption.

## THYROXINE ANALOGUES AND CHOLESTEROL METABOLISM

*A number of thyroxine analogues were tested for their effects upon cholesterol metabolism. Diiodothyroacetic acid and diiodothyroformic acid seemed most effective without altering other metabolic functions.*

Recently, the effect of L-thyroxine on concentrations of lipoprotein and cholesterol in the serum was reviewed (*Nutrition Reviews* **17**, 303 (1959)). Thyroxine was capable of decreasing by a considerable degree the concentration of cholesterol and lipoproteins in the serum of a group of schizophrenic patients. However, it had undesirable effects upon the pulse rate and it decreased the diastolic blood pressure. Theoretically at least, some agent which could be administered in addition to a normal diet might prove to be effective in decreasing the serum concentration of cholesterol in persons found to have high values.

Unfortunately, most of the analogues of thyroxine which have been capable of alter-

ing lipid metabolism also have effects which would be undesirable in patients with coronary vascular disease. These include an increased metabolic rate, tachycardia, a rise in the pulse pressure and psychomotor stimulation. The exact mechanism of the thyroid hormone itself is not understood, but recent investigations have demonstrated that some analogues may manifest predominantly one action but relatively little of another.

In an effort to determine whether some of the available analogues of thyroxine would alter the metabolism of cholesterol without undue metabolic effects, W. R. Ruegamer, M. E. Alpert and F. R. Silverman (*Endocrinology* **66**, 160 (1960)) conducted a study

in young albino rats. The experimental design was precise and the animals were housed in an air-conditioned laboratory which facilitated accurate calorimetric studies. They were given one of three diets, a commercial dog chow, a diet high in fats and cholesterol with added cholic acid, or the same high-fat diet with an increase in the amount of protein from 16 per cent to 19 per cent at the expense of carbohydrate. The thyroxine derivatives employed were as follows: 3,5-diiodothyronine; 3,5,3'-triiodothyronine; 3,5,3',5'-tetraiodothyronine; 3,5-diiodothyroacetic acid; 3,5,3'-triiodothyroacetic acid; 3,5,3',5'-tetraiodothyroacetic acid; 3,5,3'-triiodothyropropionic acid; and 3,5,3',5'-tetraiodothyropropionic acid.

In the first experiment, 11 groups of rats with eight in each group were studied. One group was fed a commercial dog chow and the other ten groups were given the high-fat diet. Nine of these ten received one of the above-mentioned thyroxine analogues. These were given by intraperitoneal injection each day in a dose of 0.5 micromoles per 100 g. of body weight. At the end of the injection period the rate of oxygen consumption in the animals was determined and blood samples were obtained for cholesterol determinations. The livers were removed and samples taken for determination of cholesterol concentration.

In the second experiment, the quantity of protein in the diet was increased to 19 per cent, but otherwise it was identical. However, several additional analogues of thyroxine were employed in comparison with thyroxine itself. These included 3,5,3'-triiodothyroethylamine; 3,5-diiodothyroformic acid; 3,5,3'-triiodothyroformic acid; 3,5,3',5'-tetraiodothyroformic acid; and 3,5-diiodothyropropionic acid.

In the first experiment it was apparent that each of the analogues had a significant effect upon the hypercholesterolemia, and two of them, 3,5-diiodothyroacetic acid and

3,5,3'-triiodothyroacetic acid, reduced the hepatic concentration of cholesterol significantly. However, 3,5,3'-triiodothyroacetic acid caused a significant inhibition of weight gain and a significant increase in consumption of oxygen.

In the second experiment, it was evident that the increased allowance of protein resulted in a lesser degree of hypercholesterolemia. It also increased the rate of growth of the control animals. Of the various analogues used in this experiment, significant decreases of the plasma cholesterol occurred with thyroxine or with 3,5-diiodothyroformic acid. The latter also had a significant effect in decreasing the concentration of cholesterol in the liver. It did not alter the rate of weight gain or the consumption of oxygen significantly.

The authors concluded that, of the analogues tested, 3,5-diiodothyroacetic acid and 3,5-diiodothyroformic acid showed the greatest preferential effect upon lipid metabolism without significantly influencing oxygen consumption or growth of the animals.

Much remains to be learned concerning the metabolism of cholesterol and other lipids. For example, an interrelationship has been demonstrated between the thyroid hormones and androgens. L. Hellman *et al.* (*J. Clin. Endocrinol. Metab.* **19**, 936 (1959)) demonstrated that myxedematous patients excreted subnormal quantities of androsterone. Administration of triiodothyronine to these patients resulted in an increase of androsterone and had a similar effect in normal subjects. Further demonstration of the relationship between those two hormonal systems was obtained by administering androsterone to myxedematous patients, which resulted in a decrease in serum cholesterol and, in one patient, an increase in rate of oxygen utilization.

The study of thyroxine derivatives demonstrated clearly that some of the effects of thyroid hormone could be separated quanti-

tatively from others. However, it remains to be determined whether this separation will be apparent in humans, and if so, whether these substances will be harmless. It would

be of great interest to learn whether the hypocholesterolemic effect of thyroxine analogues is accompanied by an alteration in the formation and excretion of androgens.

## COMPARATIVE NUTRITIVE VALUE OF COMMON EDIBLE FATS

*There appears to be little difference in the nutritive value of most common edible fats even in prematurely weaned animals.*

A great many studies have been conducted on the relative nutritive properties of various animal fats and vegetable oils, especially in comparison to those of butterfat (*Nutrition Reviews* 1, 122, 358 (1943); 2, 165, 267, 351 (1944); 3, 271 (1945); 4, 140 (1946); 8, 232 (1950); 14, 305, 349 (1956); 16, 331 (1958)). Although the general conclusion reached in most of these studies has been that there is little difference in the nutritive value of the common fats (when compensated for digestibility), there has not been agreement on certain experimental conditions.

For example, it has been claimed (E. J. Schantz, R. K. Boutwell, C. A. Elvehjem and E. B. Hart, *J. Dairy Sci.* 23, 1205 (1940)) and denied (H. J. Deuel, Jr., and E. Movitt, *J. Nutrition* 29, 237 (1945)) that even if no difference exists in animals studied from the normal weaning age, butterfat is superior in those weaned prematurely. Moreover, there are indications that the other dietary constituents may influence the relative nutritive values of the fat (Boutwell, R. P. Geyer, Elvehjem and Hart, *Ibid.* 26, 601 (1943)).

In an attempt to resolve some of these differences, E. W. Crampton, R. K. Shaw, V. G. Mackay and D. C. Schad (*J. Nutrition* 70, 81 (1960)) have measured the nutritive value of several common fats for prematurely weaned animals. One-hundred and eight puppies, 172 pigs and 266 guinea pigs

were weaned at ten, 14 and three days, respectively, and divided into groups which were placed on low-fat diets largely composed of cereals and skim milk powder with adequate vitamin and mineral supplements and 20 per cent of 15 different vegetable or animal fats. Digestibility was determined by the chromic oxide method and records of weight and feed intake were maintained for four weeks for the other animals.

Weight gains were similar for most groups of animals of each species, regardless of the type of dietary fat. However, some growth depression was seen for pigs on the fish, rapeseed and coconut oil diets, and for guinea pigs on the hydrogenated fish oil. When the mean weight gains of the five groups of each species fed butterfat diets were compared with those of all groups on other types of fat, it was found that there was 18, 10 and 14 per cent greater gain on butterfat than on other types.

These differences appeared to be related to a greater feed intake by the butterfat groups, a finding which had been ascribed by Deuel and Movitt (*J. Nutrition* 27, 399 (1944)) to flavor preference. It is also possible, however, that these differences were related to the rapid intestinal absorption of butterfat, as reported by H. J. Thomasson (*J. Nutrition* 59, 343 (1956)). Similarly, the lower growth rates of groups on fish, rapeseed and coconut oils could be equated to lower digestibility of these fats,

in agreement with Thomasson (*loc. cit.*). Thus, when these factors were taken into consideration, no significant differences in nutritional value of butterfat as compared to other animal and vegetable fats could be observed. This conclusion was also confirmed by a comparison of feed efficiency (average gain per 1000 digested calories).

No answer was obtained in these experiments to the question of the effect of dietary carbohydrate on nutritional value of the fats, since the carbohydrate source of all groups was of mixed origin. However, it seems clear that age of weaning has little or no effect on the comparative nutritional value of the different fats.

### PLASMA AMINO ACIDS AND DIETARY PROTEIN

*A method, based on measurement of changes in plasma concentrations of amino acids following a test meal, is proposed for predicting the sequence in which the amino acids of a protein become limiting for tissue synthesis.*

The nutritive value of a dietary protein depends upon how closely the relative quantities of amino acids reaching the tissues of a subject ingesting it correspond with the relative quantities of amino acids required by the tissues. A nutritionally perfect protein would be completely digestible, would have a ratio of dispensable to indispensable amino acids identical with that required by the subject (a ratio as yet undetermined), and a pattern of indispensable amino acids that coincided with the pattern of amino acid requirements of the subject.

The last of these criteria is undoubtedly most important because a measure of the extent to which the pattern of indispensable amino acids in a protein deviates from a standard approaching the ideal gives a fairly good estimate of the nutritive value of that protein. This is the basis for the chemical score method of estimating protein quality devised by R. J. Block and H. H. Mitchell (*Nutrition Abstracts & Reviews* **16**, 249 (1946)). They used the amino acid pattern of the proteins of whole egg as a reference standard.

Recently, two attempts have been made to design amino acid patterns that would serve specifically as reference standards for human nutrition studies (*Protein Requirements*. Food and Agriculture Organization

*Nutritional Studies No. 16* (1957); *Evaluation of Protein Nutrition*. National Academy of Sciences-National Research Council Publication No. 711 (1959)). These developments have made it possible to estimate reasonably well the nutritive value of a protein from a knowledge of its amino acid composition. They have not, however, obviated the need for a rapid, simple and accurate method of determining the nutritive value of a protein experimentally.

Among the experimental methods that have been used to estimate the nutritive value of a protein (*Nutrition Reviews* **10**, 33 (1952)) are the nitrogen balance technique for the classical determination of biological value or net protein utilization (Mitchell, *J. Biol. Chem.* **58**, 873, 905 (1923)), the rat growth assay for the determination of protein efficiency ratio (T. B. Osborne and L. B. Mendel, *Ibid.* **32**, 369 (1917)), and carcass protein or moisture analyses (D. S. Miller and A. E. Bender, *Brit. J. Nutrition* **9**, 382 (1955)). The nitrogen balance technique is time-consuming and inconvenient for human studies. The carcass analysis method is applicable only to animal studies, while the protein efficiency ratio method is applicable only to growing subjects and its value is extremely limited unless the many variables that influence it are rigidly controlled (Bender, *Proc. Nutrition Soc.* **17**, 85 (1958)),

a near impossibility in studies on human subjects. Therefore, new techniques that show promise as a means of estimating directly the nutritive value of proteins for human subjects are bound to interest nutritionists (*Nutrition Reviews* 11, 347 (1953)).

An investigation by J. B. Longenecker and N. L. Hause on the relationship between plasma amino acid concentrations and the composition of the ingested protein (*Arch. Biochem. Biophys.* 84, 46 (1959)) should elicit such interest. The basic assumptions underlying the investigation are that the amino acid composition of the blood plasma of animals fed a single test meal should depend not only upon the amino acid composition of the dietary protein but also upon the rate and extent to which it is digested and upon the rate and extent to which the various amino acids are metabolized by the body. Thus plasma amino acid concentrations following a test meal should reflect the nutritional adequacy of the ingested protein.

It has long been hoped by nutritionists that plasma amino acid concentrations might be useful in assessing the nutritional status of the individual and the nutritive value of proteins. Several papers are cited by Longenecker and Hause (*loc. cit.*) indicating that plasma amino acid concentrations after the ingestion of a protein are roughly proportional to the amounts of amino acids supplied by the protein. Also, in a study of the protein-sparing effect of carbohydrate, H. N. Munro and W. S. T. Thompson (*Metabolism* 2, 354 (1953)) demonstrated that, upon administering glucose to human subjects in the fasting state, the plasma amino acid concentrations decreased approximately in proportion to the accepted amino acid requirements, suggesting that they were used for protein synthesis in these proportions.

Also, after reviewing the literature on blood plasma amino acid concentrations, H. J. Almquist (*Arch. Biochem. Biophys.* 52, 197 (1954); *The Amino Acid Handbook*,

p. 152. C. C. Thomas (1956)) concluded that the amino acid pattern obtained after the ingestion of a protein frequently deviated from that expected. He noted, in particular, that the concentration of an amino acid which was deficient in the ingested protein was usually inordinately low in the plasma. He suggested that these deviations could be explained if the amino acids were used in proportion to the requirements and that the amino acid pattern of the plasma would resemble that of the protein ingested only if the protein were of high nutritive value. From these observations he concluded that the plasma amino acid pattern might provide a sensitive index of protein quality.

However, until the publication of the paper by Longenecker and Hause, no method had been devised for relating plasma amino acid concentrations to the composition of the ingested protein in a way that might prove useful for estimating the nutritive value of a protein.

The experiments from which their method was developed were performed on adult mongrel dogs weighing about 10 kg. Food was withheld from them for 18 hours; then they were fed 200 g. of a test diet containing 32 per cent protein (wheat gluten, gelatin or casein), 10 per cent cottonseed oil and 58 per cent sucrose. Immediately before the meal and at hourly intervals for five hours afterward, 25 ml. of blood was removed from each animal. The erythrocytes were centrifuged off and the amino acid composition of a protein-free filtrate of the plasma was determined by ion exchange chromatography, except for tryptophan which was determined by microbiological assay. Nine trials were run, three with wheat gluten, two each with gelatin and casein, one with wheat gluten supplemented with lysine, and one with gelatin supplemented with tryptophan.

The concentrations of the essential amino acids in the plasma of each dog at each hourly interval were tabulated, but no consistent relationship between the amino acid

composition of the protein ingested and that of the blood plasma was evident. In an effort to bring some order out of what appeared to be chaos, the authors calculated what they called "plasma amino acid ratios." This was done by averaging the five hourly plasma values for each amino acid and subtracting the corresponding fasting value to give a measure of the change in the plasma concentration of each amino acid following the ingestion of the test meal. Each of the values so obtained was divided by the corresponding value for the requirement of the dog, and this multiplied by 100 was taken as the plasma amino acid ratio for that amino acid.

For example, after the ingestion of wheat gluten, the average plasma concentration for leucine was 3.07 mg. per 100 ml. Subtracting the fasting value of 1.05 gave a value of 2.02 for the change in concentration. This divided by 8.5 (the requirement of the dog for leucine being 8.5 g. per 16 g. of nitrogen) and multiplied by 100 gave a value of 23.8. The values obtained for different amino acids in the various experiments ranged from -65 to 58.

It was assumed that the most limiting amino acid would yield the smallest positive or the largest negative number and that a tabulation of the plasma amino acid ratios in ascending order would reveal the sequence in which the amino acids of the test protein became limiting for the dog. The sequences obtained for the more limiting amino acids of gelatin and wheat gluten corresponded fairly well with the sequences calculated by the chemical score method. The sequences for the less limiting amino acids of these two proteins and for casein did not show such close correspondence.

The lack of correspondence should not be taken as a severe criticism of this procedure because there is no evidence that the sequences obtained by the chemical score method provide a reliable standard for comparison. In fact, the greatest obstacle to the development of this method is the

lack of information which will permit an estimate of its reliability. The final proof of the reliability of such a method is the accuracy with which it can be used to predict the order in which amino acids must be added to a diet containing a low level of the protein in order to stimulate growth or increase nitrogen retention.

If the method provides an accurate estimate of the sequence in which the amino acids in a protein become limiting, it should be possible to show, by using protein-depleted dogs fed a diet containing a low level of protein, that nitrogen retention increases stepwise as the essential amino acids are added one after another in the sequence predicted from the estimated plasma amino acid ratios. For the growing animal, it should be possible to demonstrate stepwise increases in growth rate using the same procedure.

Many questions can be raised about factors that might influence the reproducibility of the plasma amino acid ratios determined by the method of Longenecker and Hause; for example, the effect of the type of diet on the rate of stomach emptying (C. Peraino *et al.*, *Canad. J. Biochem. Physiol.* **37**, 1475 (1959)), the effect of changes in the rate of blood flow, the influence of rapid protein synthesis in the growing animal, and the effect of inaccuracies in the estimated amino acid requirements of the subjects. The averaging of five hourly samples undoubtedly helps to remove much of the variability, and further investigation of this technique will answer many of these questions.

In the meantime, the method should be studied carefully because it offers promise as a procedure for determining directly the sequence in which the amino acids of a protein become limiting for growth and maintenance, and thus offers promise as a direct method for estimating the nutritive value of proteins for human subjects. With further development, it may prove of value as a technique for determining individual amino acid requirements.



## NOTES

### Possible Growth Inhibitor in Uncooked Peas

E. W. Kienholz, L. S. Jensen and J. McGinnis have reported "Improvement in nutritional value of peas by cooking" under this title (*Proc. Soc. Exp. Biol. Med.* 102, 35 (1959)). They point out that field peas are deficient in methionine, but that chicks fed diets containing abundant peas do not grow maximally even with added methionine. Their studies were designed to discover the cause of the deficient growth.

Alaska peas were ground, mixed with water, autoclaved and then oven-dried at 60°C and reground. Autoclaved peas were similarly prepared but without water. Uncooked frozen peas (Thomas Laxton variety) were oven-dried at 60°C.

The authors fed diets containing high levels of peas, with added methionine, vitamins and minerals, to day-old chicks and turkey poults for two weeks. The growth rate of the turkeys was greatly increased by cooking the peas, especially with water, and was comparable to that on the control corn-soy diet. Increasing the caloric content of the diets by adding tallow had less effect than cooked peas in a diet of fewer calories.

Kienholz, Jensen and McGinnis conclude that heat inactivates a growth inhibitor in peas. They indicate that it is not a trypsin inhibitor nor is it beta-aminopropionitrile. However, they report only preliminary attempts to concentrate the material.

It is unfortunate that no record was made of dietary intake, which was ad libitum; thus the growth value of different diets is difficult or impossible to assess. Also, no pathologic studies were performed. Interference with growth is a gross result compared with histologic changes, which often provide a clue to the nutritional mechanisms involved.

One looks to future work to compare the growth value of isocalorically consumed diets, to define the lesions if any, and to

fractionate and identify the growth inhibitor if such it be.

### Nutritional Excess in Infancy and Childhood

Parents of today are exhorted on television and in the press to feed their children vitamins, minerals, meat, milk, cereals and many other items. W. A. Cochrane (*Canad. Med. Assn. J.* 81, 454 (1959)) points out that physicians have done little to restore reason, especially with respect to infants. It is thought generally that a growing baby is a healthy baby, equating size with health. However, according to M. L. Johnson, B. S. Burke and J. Mayer (*Am. J. Clin. Nutrition* 4, 231 (1956)), 10 per cent of all American children are overweight. H. V. Merideth (*Am. J. Dis. Child.* 62, 909 (1941)) found that infants of one year were 7 per cent taller than those of the nineteenth century, and boys of nine to 14 years were 6 to 8 per cent taller and 12 to 15 per cent heavier.

Maximal rate of growth may not be equivalent to optimum health and greatest longevity, if information from animals proves to be applicable to man. For example, increasing dietary protein of young rats results in an earlier appearance of kidney lesions (G. C. Kennedy, *Brit. Med. Bull.* 13, 67 (1957)).

A possible detrimental effect of excess is seen in Great Britain and Switzerland where a condition has appeared in infants known as "idiopathic hypercalcemic syndrome". These children grow rapidly but develop constipation, vomiting, abnormal renal function, and hypertension. They have been fed foods containing large quantities of vitamin D, and have elevated concentrations of calcium in their serum. British cod liver oil contains twice as much vitamin D as ours, and their fortified milk three and one-half times as much (R. Lightwood *et al.*, *Brit. Med. J.* 2, 149 (1956)).

These considerations seem to indicate that any changes which we might propose in our dietary routine must be evaluated fully before they are advocated for general adoption.

### Bisalbuminemia

The presence of abnormal proteins in the blood is being reported more and more frequently as methodology becomes more sensitive and definitive. G. Franglen *et al.* (*Lancet* I, 307 (1960)) have recently reported the presence of a second albumin in the serum obtained from a 16-year-old boy who had an unusual dermatological condition of his hands. This albumin, called a bisalbumin, migrated on paper under an electric potential a bit slower than normal albumin. However, it moved faster than the gamma-globulin. The boy's hands were cold and blue even in summer and had a horny layer over the knuckles. Also he had an inability to straighten the fingers involved. No acrosclerosis or any form of Raynaud's disease was present. The authors concluded that this was an hereditary albumin abnormality, since such conditions were also noted in the hands of the boy's father and his paternal grandfather was reported to have cold blue hands. The abnormal protein was also found in the blood of four of the patient's relatives.

The investigators believed the abnormal protein to be an albumin since zone electrophoresis on paper, cellulose acetate, and agar revealed well-defined double bands which migrated similarly to albumin and faster than the globulins. Also the alpha-globulin could be identified as clearly separated from the abnormal albumin. Secondly, using moving boundary analysis, a well-defined hump could be seen on the posterior slope of the peak and the alpha-globulin area was

clearly visible behind it. Thirdly, dyes believed to couple specifically with albumin were found to be bound to both of the bands noted. Finally, using electrophoresis on starch blocks, the two bands were shown not to differ immunologically from normal human albumin.

The abnormal albumin represented about 50 per cent of the total albumin and this proportion was remarkably constant. In fact, either the members of the family had a level of approximately 50 per cent of the total serum albumin or the abnormality did not appear at all in the blood. C. G. Bergstrand and B. Czar (*Scand. J. Clin. Lab. Invest.* 9, 277 (1957)) have reported the presence of a protein which migrates between the albumin and alpha-globulin in the serum of fetuses obtained from legal abortions up to the nineteenth week. This component amounted to about 10 per cent of the normal albumin level. However, the present investigators suggest only tentatively that the abnormal albumin they detected may represent a persistence of fetal type albumin, even though it was considerably higher in concentration than that reported by Bergstrand and Czar.

While these authors were tempted to correlate the presence of the unusual skin condition with the protein abnormality, they noted that four other members of the family, two males and two females, had the abnormal albumin but did not show any dermatological defect.

With better techniques of fractionating proteins becoming available, various hereditary abnormalities not previously observed should be detected. Whether or not these produce changes in the body's economy still has to be decided.



# NUTRITION REVIEWS

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## NUTRITION PROBLEMS AND PROGRESSIVE PATIENT CARE

The subject of nutrition cuts across many disciplines, from the technical sciences of chemistry and physiology to such social sciences as psychology and sociology. This paper considers the area where nutrition and philosophy of patient care overlap. It is often overlooked that the convalescent patient, hospitalized for whatever reason, has certain nutritional requirements, the fulfillment of which are necessarily associated with the general philosophy of hospital care. Just as the daily environment of the normal individual affects his nutritional state, so the hospital environment and approach to patient care affect the nutritional state of the convalescent.

Although the science of medicine and subsidiary fields have advanced tremendously in the past 50 years, change in the function and approach of the hospital in providing a therapeutic environment for optimal patient care facilities has not advanced as rapidly. In spite of the modernization of hospital facilities brought about throughout the country by the efforts of interested parties, particularly the influence of the Hill-Burton Plan, the philosophy of hospital care has, until quite recently, undergone very little change in the past half decade. The community hospital, however, has tended to become more and more the center of medical care, and the physician (who 25 years ago used the hospital primarily for its operating room facilities and for controlled room and board) now finds himself in nearby offices, utilizing the hospital's laboratory and x-ray facilities, its opportunities for education and teaching, and practicing medicine in an increasingly hospital-center-oriented environment.

Coincident with this tendency towards centralization of medical facilities is an increasing interest in the problem of optimal

care facilities for the patient. This has resulted in the formulation of the concept of "Progressive Patient Care," which may be described as the tailoring of the services provided by the hospital to the particular requirements of the patient (J. C. Haldeman, *Elements of Progressive Patient Care*. U. S. Public Health Service (1957)). The average ward or service in a general hospital is occupied by patients varying from the critically ill, requiring maximum attention and access to facilities, to the ambulatory patient, requiring primarily merely minimal housekeeping services. The effect of this type of organization on the efficiency and smoothness of hospital operation requires no comment; the effect of this on the patient warrants some consideration.

The Progressive Patient Care hospital customarily divides the care requirements into three categories: Intensive Care, Intermediate Care and Self Care, and provides facilities tailored to each of these requirements. A patient requiring constant attention is placed in a facility tailored to that end; generally there are one or more nurses for a minimal number of patients (four to eight), who remain at all times in sight of the patients. Oxygen, suction, special drugs and equipment are immediately available.

When the patient no longer requires these facilities he is moved to the Intermediate Care area, remaining there until either discharge or transfer to the Self Care area (J. C. Haldeman and F. G. Abdellah, "*Concepts of Progressive Patient Care*", *Hospitals* **33**, nos. 10 and 11 (1959)). It is this last area that is particularly interesting from the standpoint of nutritional therapy and instruction.

The largest number of complaints in a hospital environment involves dietary logistical problems. (One of the loudest com-

plaints concerns the temperature of the coffee.)

Two case histories of recent date are pertinent. The first concerns a Mr. W., hospitalized with a textbook case of diabetes. During his two-week stay in the hospital, the problem of diet was not brought up. Towards the end of the second week, his wife was called in, handed a pamphlet supposed to answer any questions she had, and dismissed. Mrs. W., being a bright person, augmented her knowledge in the area from other sources; thus the probability of readmission of Mr. W. is low.

The second case, Mr. G., was admitted on the diagnosis of coronary occlusion; he was found to be hypertensive. On discharge, his wife was instructed by the physician to "keep him on a salt-free diet." That statement constituted the sum total of instruction.

These two histories do not represent indictments of either the hospital care or the physician's responsibilities; nor do they represent isolated incidents in 50-bed hospitals in East and West Podunk. One case occurred in a major research center and the other in a large and technologically up-to-date community hospital. They do, however, represent a need for education in the nutritional area for those cases suffering from diseases of nutritional significance.

The towns of Podunk referred to above were not chosen ill-advisedly; where Podunk once existed now stands the town of Manchester, Connecticut, having one of the first and most successful Progressive Patient Care hospitals in the country (The Manchester Memorial Hospital). Largely under the auspices of the Division of Hospital and Medical Facilities of the United States Public Health Service of the Department of Health, Education and Welfare, this hospital has provided laboratory facilities for research in operational and dietary problems, nursing facilities, and in patient attitudes.

The Self Care area in this hospital provides a suitable environment for education

of the patient in the nutritional problems he may be expected to face on discharge. The area is for the most part home-like with sitting rooms and private kitchen for breakfast and snacks, and the patients are treated more like guests than hospitalized convalescents. They are expected to report for treatment and for laboratory work and they eat in the hospital cafeteria. Each patient has a slip noting his dietary restriction or requirements, and presents this at the cafeteria at meal times, but in most cases he may still select his food with some degree of choice.

Most important is the opportunity provided for the patient (and spouse) to discuss, understand, and receive instruction on the dietary problems to be faced on discharge. Basically, the Self Care unit provides an optimum medium for dietary and nutritional control and education. It also provides the hospital with the physical facility for dietary and nutritional education on a larger scale than is usually available. Thus physicians can refer a non-hospitalized patient with problems of nutritional significance to this care area for instruction and control without placing a further burden on the nursing and medical facilities or unnecessarily isolating and bedding down an ambulatory patient.

It has been noted (A. Meredith, "*Nutritional Counseling for Patients in a Community.*" *J. Am. Dietet. Assn.* **33**, 108 (1957)) that the mere availability of nutritional counseling facilities in a community does not guarantee the use of these facilities. However, if such facilities could be hospital-center oriented within the framework provided by Progressive Patient Care, the effectiveness of such counseling and education could be markedly increased.

The extension of the Progressive Patient Care concept to a fourth level, Home Care, would provide a further means for education and control of nutritional cases. In many communities today the visiting nurse represents a further extension of the concept of tailoring care requirements to the needs

of the patient. This hospital-center-oriented home care is enjoying increasing popularity and provides an excellent medium for nutritional therapy. It may be anticipated that in the future it will be an integral part of any system of Progressive Patient Care.

A problem constantly faced by physicians is that of assuring that his patient receives the basic nutritional requirements for sustenance and tissue repair through convalescence. A patient assigned to a bed in a service with a fairly high noise level, minimal attention, a roommate who may be much sicker, faced with unfamiliar or unpleasant hospital odors and a decor that is charitably described as neutral, is not to be expected to maintain a normal food intake, and admonitions to eat have little value. Most attempts to solve this problem have been in terms of making the food more appetizing, of greater variety, or more decorative. This approach, although admirable and necessary, ignores the effect of the environment upon the patient.

A generalization of a type of case not infrequently encountered in community general hospitals may exemplify the effect of a dynamic hospital environment. In the conventional hospital, a 65-year-old woman with a broken hip usually undergoes orthopedic surgery and is then bedded down for a relatively long period of time, depending on the home care facilities available. In the Progressive Patient Care environment, on the other hand, the same patient may be in the Intensive Care area for a brief period and then move to the Intermediate Care area. As soon as the patient is able to cope for herself with minimal assistance, she is moved to the Self Care area, an environment much better suited to preparing the patient for return to the non-hospital environment than is the conventional hospital room.

Several aspects of this moving through various care areas are pertinent. First, the patient is made aware of convalescent progress; second, the change in environment as this progress takes place is better fitted to the patient's requirements; and third, the

patient is slowly and unobtrusively made self-sufficient and released from dependence on hospital facilities. The interaction between these aspects of a Progressive Patient Care system and the patient's dietary and nutritional problems cannot be understated, since the patient-environment relationship is one of the primary factors in maintaining the nutritional requirements for convalescence.

The type of service rendered by the Nutrition Clinic of the Johns Hopkins Hospitals, serving both inpatient and outpatient populations, is not available to the relatively small, general community hospital. At this clinic, counseling and guidance, even food-shopping assistance, is provided patients suffering from nutritional problems of one sort or another. However, similar facilities can become available to the small hospital through provision of a Self Care area as in the Progressive Patient Care hospital. Thus the small community hospital can operate a service (previously enjoyed only in the metropolitan hospitals), which, with the cooperation of the physician, the dietitian and in some cases, the social worker, tailors the patient-care facilities to the needs of the patient.

To conclude, the logistical aspects of hospital nutrition should be re-examined in the light of the Progressive Patient Care concept. Many patients requiring Intensive Care facilities place no demand on the dietary facilities of the hospital, while others in the same area require special dietary consideration consistent with the etiology of hospitalization. The Intermediate Care area poses much the same problems as a conventional hospital, excepting the absence of the seriously ill and the ambulatory. The Self Care area, as noted above, is characterized by the patient going to the food rather than vice versa. Thus the concept of Progressive Patient Care calls for a different approach to the logistics of food preparation, control and supply than is presently maintained by the conventional hospital, and provides a basis for revamping hospital

dietetic philosophy (A. C. Donovan and B. Meyer, "A New Challenge to Hospital Dietitians, *Progressive Patient Care*." *J. Am. Dietet. Assn.*, in press (1960)).

The Progressive Patient Care concept is being adopted increasingly throughout the country. With its adoption, an opportunity is provided for improved community nutritional counseling, improved patient educa-

tion, better hospital facilities for patients with problems of nutritional import, and an environment tailored to assure the nutritional requirements for convalescence.

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## DIET AND PREGNANCY

*The nutrient intakes of a large percentage of the primigravid women during the seventh month of pregnancy were below the recommended dietary allowances, yet the course of pregnancy and condition of the baby were normal.*

A number of studies within the past few years have indicated that the diets of pregnant women can deviate from recommended allowances without producing any great disturbance either in the course of pregnancy or in the condition of the infant (*Nutrition Reviews* 12, 260 (1954); 16, 6 (1958)). It is, however, recognized that extremely poor diets such as those associated with intakes of ascorbic acid of 20 mg. per day or less will have deleterious effects on the course of pregnancy (*Ibid.* 16, 6 (1958)). The important problem is how far below recommended allowances can the intake go during this critical period without producing physiological disturbances either in the mother or in the infant.

A. M. Thomson (*Brit. J. Nutrition* 12, 446 (1958)) claims that "despite extensive investigation it remains uncertain whether diet taken by a pregnant woman exerts an important influence upon the clinical course and outcome of her pregnancy." For this reason he started to gather dietary and clinical histories of pregnant women in Aberdeen.

Before the study was initiated, he considered how he might best secure valid information on food consumption from his patients. The limitation of any questionnaire technique was vividly revealed when the food fed to eight scientists living in a residential club was carefully measured during a 24-hour period and then, without prelimi-

nary notice, each man was asked to recall in detail what he had eaten (S. D. Morrison, F. C. Russell and J. Stevenson, *Brit. J. Nutrition* 3, V (1949)). When the men demonstrated the quantities they had consumed with actual food samples, the recollected food intake was, in almost every case, an underestimate when compared with the actual weighed portions that had been served.

To overcome the above limitation, Thomson decided to secure his dietary information by having the pregnant women carry out a one-week weighed dietary survey in their homes. Subjects for the study were limited to primigravida (women who are pregnant for the first time), since these individuals would be less preoccupied with domestic responsibilities and less subject to complications. Moreover, in Aberdeen about 90 per cent of these women use the hospital for their confinement.

During 1950-51, every sixth married primigravid woman who attended the antenatal clinic in Aberdeen was selected for the study. The number of women from the poorer social classes was increased in the subsequent two years by including every primigravida whose husband's occupation was semi-skilled or unskilled. The dietary record was made during the seventh month of pregnancy, since by that time the alterations in eating associated with nausea and



vomiting have fairly well subsided. Furthermore, the fetus is not so large that it would alter the functioning of the gastrointestinal tract and thus change eating habits.

Acceptable dietary histories were secured from 489 of the 713 subjects. The percentage of "reliable" histories decreased from 93 in the top social group to 61 in the lowest group. The calculated nutrient intakes provided by these diets suggested a slight reduction in mean caloric intake from 2633 in the top social group to 2354 in the lower group. Moreover, the mean values for all groups were below the caloric intake of 2750 recommended by the British Medical Association (*Report of the Committee on Nutrition, London (1950)*). The protein intake showed a similar reduction from 80 to 72 g. respectively for the social groups. The other major differences in mean intakes were in calcium, where the top social group secured 1.19 to the lower group's 0.88 g.; and in ascorbic acid, where the values progressively decreased from 79 to 61 mg.

Within each group there were fairly large percentages of women whose intakes did not meet those recommended by the British Medical Association. Eighty-three per cent in the top social class and 92 per cent in the lowest social group did not meet the 96 g. of protein intake recommended by the Association. Similar percentages existed for the women who did not meet the recommended calcium intake. For the other nutrients, such as vitamin A, riboflavin and niacin, one quarter to one half of the women did not meet the recommended allowances. In all cases, a larger percentage of the women in the upper social class attained the recommended allowances.

To determine to what extent the diet records represented the food habits of the subjects, a second survey was undertaken six weeks later for 11 of the subjects. The results agreed exceedingly well with those of the first survey.

Thomson (*loc. cit.*) concludes his first report with the statement that "it seems clear that patients differ widely in the

amounts and nutritive values of the diets they take during pregnancy. The question is whether these variations have any clinical significance."

Thus a second paper by Thomson attempts to evaluate the effect of dietary intake on pregnancy with respect to the 489 subjects described above (*Brit. J. Nutrition* 13, 509 (1959)). Normal pregnancies were defined as those in which there was no specific abnormality requiring treatment; labor was completely spontaneous within 24 hours; the baby was in good condition and weighed 6 pounds or more at birth; and the mother and baby were discharged together from the hospital, with the baby receiving nothing other than breast milk.

For the 489 subjects, 197 experienced a normal pregnancy according to the above definition, whereas 292 were abnormal. The women in the abnormal group had greater mean caloric intakes. Associated therewith were slightly greater intakes of most of the nutrients. A partial explanation for these higher intakes, according to Thomson, is the fact that those who developed pre-eclampsia were usually those who had gained weight excessively. This, however, does not completely explain the difference, since only a small percentage of the women were pre-eclamptic. From an extensive statistical evaluation of his data, Thomson suggests that "the present findings indicate that overeating may play some part in the etiology or the development of pre-eclampsia. But a conclusion that overeating is a major cause would probably not be warranted." The latter statement is based on the fact that there was a wide range of caloric intake and of weight gain among both the pre-eclamptics and the women who went through a normal pregnancy.

There was no relation between caloric intake and length of the gestation period. The birth weight of the baby tended to increase with increase in caloric intake. This was true within each social class, but there was evidence that social class had a greater influence on birth weight than caloric intake.

The women in the upper social classes were taller and heavier, and this may have accounted for the fact that their babies weighed more at birth. At any rate, the correlation coefficients for these factors were small. There was no indication that the birth weight was associated with any of the other nutrients to an extent greater than that found for calories.

The women whose babies were delivered by Caesarean section had lower caloric intakes than the other women. This is probably associated with the fact that the smaller women had a relatively high incidence of contracted pelvis and thus required Caesarean section.

Among the 489 single births, there were 14 cases of either still births or deaths during the first week of life. The diets of the mothers of these babies did not differ appreciably from those of the normals. Thomson points out that one of the shortcomings of his study may have been the small number of subjects examined. One of the prominent changes in maternal health during World War II was the reduction in the still-birth rate in England and Wales from 38 per 1000 in 1940 to 28 in 1945. This reduction he attributed to the improved nutrition of pregnant women. In order to see an improvement of this magnitude as a result of improved diet, Thomson estimates he would have needed 10,000 subjects.

The incidence of breast feeding at the time the mothers left the hospital was the same for all three social classes. Thomson points out that if superior social circumstances, including superior diet, had any effect on lactation, this would be counter-

balanced by the adverse effect of the older age of the upper social group. (The average age of mothers in the lower group was 22, but was 28 in the upper social class.) F. E. Hytten, J. C. Yorston and Thomson (*Brit. Med. J.* 1, 310 (1958)) indicate that the most important factor in maintaining breast feeding for at least the first three months of lactation is the maternal attitude.

Thomson concludes his report with the statement that "these epidemiological findings underline the difficulty of demonstrating the influence of nutrition on human lactation in western civilization." Moreover, in reviewing at some length the various nutritional studies that have been made over the past thirty years among pregnant women and attempts to relate nutrition to the course and outcome of pregnancy, he summarizes that "the results of all these studies have been substantially negative; even though some of the authors have made the most of minor and inexplicable correlations, or have argued that their technique must have been inadequate."

Although these studies suggest that the diets of pregnant women can show considerable variation both in quantity and nutritional quality without clinically detectable damage to either the mother or the child, they do not necessarily indicate that nutrition has no impact on pregnancy. As Thomson points out, physiological adaptation of both mother and child may overcome some of the dietary shortcomings. Furthermore, the dramatic reduction in still births seen in Britain in World War II serves as a spectacular natural experiment indicating the profound effect that improved nutrition can have on the course of pregnancy.

### HYPOGLYCEMIA AND HEART DISEASE

*Hypoglycemic reactions to insulin in a group of coronary patients were prevented by potassium chloride. Phentolamine had no effect. Such reactions apparently are due to hypokalemia.*

For many years clinicians have recognized that hypoglycemic reactions must be avoided

in elderly diabetic patients because of the great risk of myocardial infarction. Most

have accepted this dictum without concerning themselves with the mechanism of damage to the coronary circulation. Those who have studied it have attributed the effect to a secondary release of epinephrine after symptoms of hypoglycemia occur. They have found tachycardia, systolic hypertension and an increase in cardiac work, coupled with electrocardiographic changes consisting of widening of the QRS interval, minor decreases in the P-waves, depression of the ST segment, and flattening or inversion of the T-waves (H. Blotner, *New Engl. J. Med.* **203**, 709 (1930); R.A. Gilbert and J.W. Goldzieher, *Ann. Int. Med.* **25**, 928 (1946); and B. Gandevia, *Med. J. Australia* **1**, 33 (1954)).

Because of the frequency of coronary sclerosis in patients with diabetes mellitus, E. S. Egeli and R. Berkmen (*Am. Heart J.* **59**, 527 (1960)) decided to study hypoglycemic reactions in a group of nondiabetic patients who had coronary arterial disease. They selected 16 patients who had angina pectoris and 22 patients who had had myocardial infarctions in the past, and these 38 patients were then divided into four groups. After the administration of the test substance, blood was drawn at intervals of 15 minutes for a total of two hours and determinations were made of the sodium, potassium, calcium and glucose content. Electrocardiograms were taken at the same intervals.

The first group of 14 subjects was given an insulin tolerance test (0.2 units per kg. of body weight). The second group was given the same quantity of insulin but was given potassium chloride either orally in a dose of 5 g. before the administration of insulin or by intravenous injection in the amount of 2 g. after the effects of insulin had become apparent. A third group of eight patients was given the same amount of insulin, but in addition was given phentolamine 30 to 45 minutes later. The fourth group of patients was given an injection of epinephrine alone.

The results confirmed the observations of other groups (A. Keys, *Am. J. Physiol.* **123**, 608 (1938)). The patients developed clinical and biochemical manifestations of hypoglycemia and their electrocardiograms showed changes, primarily in the ST segments and T-waves. Measurements of potassium, sodium and calcium disclosed a marked hypokalemia, a mild hypernatremia, and no significant change in the calcium. In the second group, administration of potassium chloride afforded a remarkable degree of protection against the effects of hypoglycemia. The electrocardiographic changes were prevented and there was some inhibition of the hypoglycemic effect of insulin. In three instances, hypoglycemia was allowed to develop before potassium chloride was given by injection, whereupon the electrocardiographic changes were reversed promptly. The third group developed changes similar to those of the first and phentolamine gave no apparent protection. The fourth group developed arrhythmias and changes in the ST segment and T-waves similar to those seen in group one.

Most investigators have contended that the electrocardiographic changes found in a hypoglycemic patient are due primarily to the release of epinephrine. Hypoglycemia has been known to induce hypokalemia and mild hypernatremia, but these changes were not considered to be of great significance. A little known but previously reported effect of potassium administration is its ability to elevate the concentrations of glucose in the blood, presumably through glycogenolysis (C. A. Ashford and K. C. Dixon, *Biochem. J.* **29**, 157 (1935)). This effect was confirmed in the present study, but it did not entirely prevent a decrease in blood sugar.

Phentolamine, which inactivates pressor amines such as epinephrine, did not prevent the electrocardiographic changes associated with decreases in the blood concentrations of glucose and potassium. Moreover, the electrocardiographic abnormalities induced by epinephrine were accompanied by a rise

in blood sugar. Thus from this information one may conclude that the electrocardiographic abnormalities of hypoglycemia are not related to the level of glucose in the blood, but are directly related to a decrease in the concentration of potassium in the blood. Although epinephrine produced similar changes, the fact that phentolamine did not prevent their occurrence would suggest that the changes in the electrolytes were the most important.

This study was astutely planned, well executed and carefully presented. It is of

interest to recall that J. M. Bryant (*Proc. Soc. Exp. Biol. Med.* **67**, 557 (1948)) reported the effect of orally administered potassium salts on the electrocardiogram of patients who had hypertensive cardiovascular disease. The familiar pattern, which has been termed "left ventricular hypertrophy pattern", was altered toward normal by the administration of potassium.

This study may reactivate interest in the significance of electrolytes with respect to both experimental and clinical heart disease.

### DIETARY FAT AND HUMAN MILK

*Up to a certain point, increases in dietary fat in the human female are accompanied by increases in milk fat, and also by increases in lipases, esterases and alkaline phosphatases.*

It has previously been shown that increases in dietary fat are correlated with increases in the fat content of human milk (M. G. Karmarkar *et al.*, *Lancet* **2**, 909 (1958)). Suggestions have also been made that milk contains certain lipases (D. Jacqmain and M. Loncin, *Proc. Thirteenth Internat. Dairy Congress* **2**, 368 (1953)) and phosphatases (E. Chanda and E. C. Owen, *Brit. J. Nutrition* **5**, 228 (1951)), both of which may play important roles in the health and nutrition of newborn infants. R. A. Stewart, E. Platou and V. J. Kelly (*J. Biol. Chem.* **232**, 777 (1958)) have further suggested that alkaline phosphatase in human milk is related to the fat content of the milk.

With these observations in mind, Karmarkar and C. V. Ramakrishnan have attempted to determine whether correlations actually exist between dietary fat and milk fat and whether the fat content of milk affects the concentrations of lipases, esterases, alkaline phosphatases and acid phosphatases (*J. Nutrition* **69**, 274 (1959)).

Subjects for the investigation were 60 lactating women of normal health living in and around Baroda, India. The subjects

were chosen to represent the same lactation period, namely three to four months, because of the possibility of enzymatic changes during the lactation period. The daily diet of the individual subjects was ascertained by collecting equal amounts of all the food-stuffs and supplements consumed by the subject. In the analysis of this food, a separate weighing was first made of each food-stuff; then all the foods were thoroughly homogenized. A portion of the homogenate was dried in an electric oven at 90°C for 12 hours so that the fat content could be measured. These analyses were carried out for three separate days so that an average daily intake of fat could be estimated.

Samples of milk for analysis were collected between the noon and night feeding (about 3 p.m.) by voluntary expression. Lipase was estimated according to the method of R. A. Boissonnas *et al.* (*Helv. Chim. Acta* **31**, 1571 (1948)). Esterase was estimated by essentially the same method as that employed by C. J. Harrer and C. G. King (*J. Biol. Chem.* **138**, 111 (1941)). Acid and alkaline phosphatases were estimated by the method of R. K. Morton (*Methods in Enzymology*, Vol. II, p. 427. Academic Press, Inc., New York (1955)).

For analysis of results, the women were divided into four nearly equal groups on the basis of their daily intake of fat (group one, 8.0 to 27.5 g.; group two, 27.5 to 50.0 g.; group three, 50.0 to 72.0 g.; and group four, 72 to 115.0 g.). Although there was a slight significant increase in fat content of the milk as the dietary fat was increased, this increase reached a maximum in the third group. On the other hand, mean activity of the milk for total lipase, total esterase and alkaline phosphatase showed increases of as much as 25 per cent from the first to the last group. Acid phosphatase activity of the fourth dietary group was decreased to nearly half that of the first group.

Although the authors contend that these values show a high correlation between the

fat content of milk and the activity of all the enzymes tested, with the exception of the acid phosphatases, it would appear that a closer relationship exists between the fat content of the diet and the increase in enzyme activity. Thus it cannot be said that the stimulus for increase in enzyme activity is due to the fat content of the milk.

A very good negative correlation exists between the fat content of the diet and the mean acid phosphatase activity, but the correlation is much weaker between the fat content of the milk and this activity.

The authors add that unpublished observations indicate that neither dietary protein nor milk protein has been found to affect the activities of the above-mentioned enzymes.

### SPOT TEST FOR CERULOPLASMIN

*Ceruloplasmin deficiency occurs regularly in subjects who develop Wilson's hepatolenticular degeneration. A simple spot test facilitates early recognition, permitting preventive treatment.*

As our understanding of diseases improves, the classical syndromes are increasingly redefined as advanced or gross manifestations of biochemical or enzyme abnormalities formerly not recognized. Means of testing for these aberrations then becomes important for diagnosis and thus prevention of the fully developed clinical syndrome.

Hepatolenticular degeneration, or Wilson's disease, is typical of such a situation, and a simple spot test for ceruloplasmin has now been devised as a screening test for subjects with pre-clinical Wilson's disease.

The underlying biochemical abnormality in this condition, while not entirely agreed upon, evidently relates to a genetically determined defect of copper metabolism. Progressive tissue deposition of the metal leads to the eventual liver cirrhosis and damage to the lenticular nucleus called Wilson's hepatolenticular degeneration. The etiology of Wilson's disease has been reviewed in this journal (*Nutrition Reviews* 16, 37(1958)) and,

more recently, in an editorial by J. M. Walshe (*Ann. Int. Med.* 51, 1110 (1959)). Whether inability to form ceruloplasmin, the copper transport protein of the plasma, is the primary defect is not proved, as not every case of Wilson's disease shows this deficiency (see below). An alternative hypothesis, that an abnormal intracellular protein is formed with increased affinity for copper, has its supporters. Walshe discusses these theories.

Incidentally, the thesis that ceruloplasmin is in several globulin fractions (alpha, beta and gamma) has been discredited by J. N. Cumings and C. J. Earl (*J. Clin. Path.* 13, 68 (1960)). Using starch gel electrophoresis with suitable staining, they demonstrated that ceruloplasmin is localized in Smithies' F-alpha-2 fraction. The authors conclude that globulins prepared by sodium sulfate precipitation by previous workers were not as pure as had been judged by paper electrophoresis.

Further work on normal subjects, patients

with Wilson's disease and their relatives is reported by G. E. Cartwright, H. Markowitz, G. S. Shields and M. M. Wintrobe in the twenty-ninth of their "Studies on copper metabolism" (*Am. J. Med.* 28, 555 (1960)). They outline the orthodox theory of the etiology of Wilson's disease; an individual, homozygous for the abnormal gene, cannot synthesize ceruloplasmin adequately, with resulting secondary increased absorption of copper from the gastrointestinal tract, excessive deposition of copper in tissues and increase in non-ceruloplasmin copper in the serum.

The authors show that serum copper and ceruloplasmin concentrations are not perfectly correlated with the presence of Wilson's disease and show little correlation with its severity or duration. They conclude, therefore, that "decreased concentration of ceruloplasmin is not the single uncomplicated determinant of the disease." They point out that, since the metabolic changes of Wilson's disease have become known, no case has been authenticated without the corneal copper deposits known as Kayser-Fleischer rings.

It seems reasonable to suppose that, as in other conditions, damaging and symptom-producing tissue deposits (in brain and liver) are important by virtue of their amount and duration, which might well not be correlated with circulating ceruloplasmin levels at a given time. Perhaps the Kayser-Fleischer ring is a better measure of brain and liver copper deposits as well as of those in the cornea.

Nevertheless, although ceruloplasmin's basic role in Wilson's disease is debatable, its measurement is valuable as a screening test in diagnosis. The availability of two drugs (dimercaptopropanol and penicillamine) which promote copper excretion and lead to clinical improvement makes early diagnosis important.

P. Aisen *et al.* have written a paper entitled "A rapid screening test for deficiency of plasma ceruloplasmin and its value in the

diagnosis of Wilson's disease" (*Am. J. Med.* 28, 550 (1960)). They point out that continued severe deficiency of ceruloplasmin rarely occurs except in Wilson's disease and that it takes seven or eight years of life before sufficient damage has occurred to produce clinical symptoms.

In an effort to discover children before the development of severe damage and symptoms, the authors developed a spot test for ceruloplasmin based on the ability of this substance to catalyze the oxidation of paraphenylenediamine, with formation of a purple-blue spot. The authors consider the normal plasma or serum ceruloplasmin values to be 18 to 35 mg. per cent and point out that values below 15 mg. per cent are found only in the newborn and in the nephrotic syndrome, as well as in Wilson's disease and "occasionally in unaffected relatives of these patients."

Filter paper strips were prepared by immersion in a solution of paraphenylenediamine and then drying. A drop (10 microliters) of "standard" serum or plasma was applied to the paper at each end, and the unknown sera applied in between. The standard was adjusted to contain 18 to 20 mg. per cent of ceruloplasmin. The strip was incubated in a moist flask in a water bath at 45° to 55°C for five to ten minutes and the intensity of the developed blue spot estimated in comparison to that of the standard.

The authors evaluated the specificity, sensitivity and reproducibility of the spot test by statistical means, testing 27 sera from cord blood, 133 miscellaneous sera from infants, children and adults, and sera from six patients with Wilson's disease; all the last contained less than 18 to 20 mg. of ceruloplasmin by the spot test.

Aisen *et al.* point out that children with persistent deficiency of ceruloplasmin will almost certainly develop clinical Wilson's disease in time. With the discovery of ceruloplasmin deficiency, an opportunity would exist for minimizing copper intake and in-



creasing its excretion. Thus prevention of hepatolenticular degeneration might well be achieved in such children. The spot test is equally of use in the diagnosis of adult cases with suspected clinical Wilson's disease.

The authors discuss certain unrelated conditions with low plasma ceruloplasmin values. They point out that patients with Wilson's disease may have temporarily in-

creased levels and they refer to one documented case with normal ceruloplasmin concentration and no "relevant intercurrent condition."

Aisen and his colleagues have developed a very simple test which should be of considerable screening value in the diagnosis of Wilson's disease and in identifying children before symptoms develop.

## MYOCARDIAL INFARCTION AND SIPPY DIETS

*Groups of autopsied patients were used to investigate the correlation between diet and heart disease. The study reveals a significant increase in myocardial infarcts among ulcer patients treated with the Sippy regimen.*

Persons with chronic peptic ulcers have a higher incidence of myocardial infarcts than others, according to J. N. Morris and M. D. Crawford (*Brit. Med. J.* **2**, 1485 (1958)). This increase may be due to the type of diet they consume. Milk products are commonly used and butterfat could be suspect because of its reported effect on blood coagulation (A. Keys, E. Buzina, F. Grande and J. T. Anderson, *Circulation* **15**, 274 (1957)), on clot lysis (R. F. Scott and W. A. Thomas, *Proc. Soc. Exp. Biol. Med.* **96**, 24 (1957)), on experimental coronary thrombosis and myocardial infarcts (Thomas and W. S. Hartroft, *Circulation* **19**, 65 (1959)) and on blood cholesterol levels in man (E. H. Ahrens, Jr., J. Hirsch, W. Insull, Jr., and M. L. Peterson, in *Chemistry of Lipids as Related to Atherosclerosis*, p. 222. Charles C. Thomas, Springfield, Ill. (1959)).

In an attempt to determine whether the higher incidence of infarcts is associated with the therapy, R. D. Briggs *et al.* (*Circulation* **21**, 538 (1960)) divided chronic peptic ulcer patients into two groups on the basis of the autopsy record. Group one included 97 patients who had been treated with milk or cream as in the Sippy diet, and group two consisted of 97 patients who had not used milk or cream. A third group of 194 patients without ulcers was chosen to match

the patients of each of the above groups by utilizing the next autopsy performed on a patient of matching age, sex and race. Acute peptic ulcer patients were excluded because the diet history would be lacking in most cases. If the pathological diagnosis was definite, it was used as the criterion for determining chronicity. Otherwise a statement that fibrosis was present at the base of the ulcer was required.

Autopsy and clinical records from 1940 to 1959 were examined in ten hospitals across the United States. Included in the data were site of the chronic peptic ulcer, age, sex, race, year of death, height, body weight, history and duration of diabetes, history and duration of treatment with Sippy or similar diet, principal diseases at death, and presence or absence of a myocardial infarct.

After the survey was completed, the records were divided into a Sippy ulcer group and a non-Sippy ulcer group. Patients in each group were matched according to age, sex, race, hospital, and the characteristics for each of the groups were tabulated, such as height and body weight.

An identical study was pursued in five British hospitals.

Average heights and weights differed little. Incidence of diabetes mellitus was low in all

groups and removal of these patients from the study made little difference. Complications of the ulcer were the cause of death in 34 Sippy-treated ulcer patients and 23 non-Sippy-treated ulcer patients.

There was no difference in the incidence of myocardial infarction between the ulcer group not treated with the Sippy diet and the non-ulcer group (15 per cent). In the Sippy diet group, however, the incidence of myocardial infarction was significantly higher (36 per cent).

In Great Britain, the 95 ulcer patients treated with milk had an 18 per cent incidence of myocardial infarcts. The 95 non-ulcer controls had an incidence of 8 per cent and the 190 ulcer patients not treated with the Sippy diet had an incidence of 3 per cent.

An increased incidence of myocardial infarction was associated with treatment by means of Sippy or similar diets. The role of

diet and medication is discussed by the authors, who conclude that the responsibility of butterfat is not proven by their study, but that the association warrants suspicion.

The lower incidence of myocardial infarcts among patients in England than in comparable groups in the United States is noteworthy.

The fact that 38 patients on the Sippy diet compared with only 23 on the non-Sippy diet died of complications of the ulcer suggests that those on the Sippy regimen had a more severe disease. This difference could conceivably affect the interpretation of the results. Other important factors to be considered are the presence or absence of treatment with magnesium hydroxide, the possible role of sedatives of various types, and the incidence of hypertension and/or renal disease in the various groups. This appears to be a fruitful field for further epidemiological and clinical investigation.

### PRODUCTION OF LOW-FAT MILK

*Cows receiving rations of cooked high-starch feeds or bread produced a low-fat milk. This effect was correlated with a decrease in rumen acetic acid and an increase in propionic acid.*

E. B. Powell (*J. Dairy Sci.* **22**, 453 (1939); **24**, 504 (1941)) reported that the fat content of cow's milk could be depressed by providing rations low in roughage or by feeding the roughage in a finely ground state. The fat content of the milk was, however, increased when the concentrates fed were first fermented with rumen material and a relationship was proposed between the activities in the rumen and the composition of milk. G. E. Stoddard, N. N. Allen and W. H. Patterson (*J. Animal Sci.* **8**, 630 (1949)) and C. C. Balch *et al.* (*J. Dairy Res.* **19**, 39 (1952)) noted further that the depression of the fat content of milk by low-roughage rations was associated with a lowered proportion of acetate and increased propionate in the rumen.

Because it has been difficult to reproduce these results consistently, J. C. Shaw and

co-workers (*J. Nutrition* **69**, 235 (1959)) have sought to develop rations that would do this and thus provide a firm basis for further studies of the factors that control milk fat secretion.

Three feeding trials were conducted during 1951, 1955 and 1956, with a total of 25 lactating cows. The cows were all in their second to fifth months of lactation and consisted of two Jerseys, seven Guernseys, 12 Holsteins and four Ayrshires.

In the first trial, normal feeds were employed. Four cows were maintained for two weeks on a regulated diet consisting of 15 pounds of alfalfa hay daily plus concentrate mixture number one (mainly corn meal, hominy feed, distillers' dried grains, brewers' dried grains, crimped oats, wheat bran and linseed meal). The cows were then changed to three or four pounds of hay per

day plus increased amounts of the same concentrate. No marked decrease in the fat content of the milk was achieved by increasing the proportion of the concentrate.

In a second trial, three cows receiving 14 to 22 pounds of bread daily showed a decrease of over 30 per cent in the fat content of the milk. Only slight decreases occurred in the fat content of the milk of three other groups of cows fed varying amounts of alfalfa hay plus high- and low-protein concentrates. The differences between the bread group and the other three groups were found, by analysis of variances, to be highly significant, but no significant differences were observed among the three non-bread groups (high-protein concentrate and low-roughage; low-protein concentrate and low-roughage; low-protein concentrate and 12 pounds of hay).

The actual total digestible nutrient (TDN) as calculated from F. B. Morrison's tables (*Feeds and Feeding, twenty-first edition. Morrison Publishing Co., Ithaca, New York (1948)*) were 114, 101, 107 and 11 per cent of optimum for the four groups. Average body weight changes (in pounds) during this period were +67, +51, -37 and 0. The first group (high-protein concentrate and low roughage) maintained production of milk at a somewhat higher level than the other groups, probably because of a more uniform and slightly higher energy intake. The bread ration was also found to produce a low level of butyric acid in the milk fat, but had little effect on its iodine number. There was a positive correlation between the fat content of milk and rumen acetic acid and a negative correlation between the fat content of milk and rumen propionic acid.

In a third trial, three groups of cows received rations consisting of concentrate mixtures made up of cooked or heated high-starch, low-fat feeds. Specifically, these rations consisted of 6 pounds of alfalfa hay daily per cow plus a concentrate mixture containing approximately one-third white bread and one-sixth low-fat milk for group one and a concentrate containing almost

one-half bread for groups two and three. The total digestible nutrient intakes were approximately 115, 75 and 115 per cent of optimum for groups one, two and three, respectively.

Groups one and three maintained milk production equally well, whereas that of group two fell more rapidly, probably because of the low energy or protein intake. Group three, on the low-protein and high-energy regimen, exhibited the greatest decrease in the fat content of the milk (33 to 40 per cent), whereas group two on the same ration, but on a low-energy intake, showed no decrease in the fat content of the milk. Group one, with a relatively high-protein intake, also exhibited a decrease in the fat content of the milk but much less than group three. The milk non-fat solids remained relatively uniform regardless of the diet.

The authors point out that the controlling influence on the fat content of milk is probably the synthesis of the lower fatty acids by the udder. The possible significance, in relation to the decrease in milk-fat production, of the large increase in the proportion of propionic acid and of valeric and higher acids is not known. It is believed, according to the authors, that the increased production of propionate may play an important role by virtue of its antiketogenicity, *i.e.*, by decreasing the formation of beta-hydroxybutyrate by the liver.

Shaw and S. Lakshmanan (*Atomic Energy and Agriculture Symposium, vol. no. 49 (1957)*) have proposed that the synthesis of the volatile fatty acids of milk from acetate and beta-hydroxybutyrate represents the bulk of the lipogenic synthesis of the lactating udder. The present authors, therefore, suggest that the decreased fat content of milk observed during the feeding of diets consisting primarily of cooked concentrates is due to a deficiency of both major blood precursors (acetate and beta-hydroxybutyrate) of the volatile fatty acids of milk. They believe that the decrease in acetate is due to an actual decrease in relative produc-

tion in the rumen while the decrease in beta-hydroxybutyrate is caused by the antiketogenicity of propionate.

The authors further point out that the iodine numbers of the milk fat did not change (as occurs with fasting) with these

diets. This suggests that an increase in the degree of unsaturation is not necessarily associated with changes in the fat content of the milk induced by alterations in the ration which decrease the volatile fatty acid content of milk fat.

## ACID MUCOPOLYSACCHARIDES AND ATHEROSCLEROSIS

*The acid mucopolysaccharide content of the aortas of cholesterol-fed rabbits was studied. Colloidal iron stained mild atheromatous lesions, but chemical analysis did not confirm the presence of acid mucopolysaccharides.*

Pathological deposits of ground substance have been found in cases of human xanthomatosis and experimental atheromatosis. In studying cholesterol-fed rabbits, D. Adlersberg, C. I. Wang and L. Strauss (*J. Mt. Sinai Hosp.* **24**, 655 (1957)) and Wang Strauss and Adlersberg (*A.M.A. Arch. Path.* **64**, 501 (1957)) found increased concentrations of acid mucopolysaccharide in the aorta and dermis.

E. Oppenheim and M. Bruger (*Circulation* **6**, 470 (1952)), H. H. Stumpf and S. L. Wilens (*Proc. Soc. Exp. Biol. Med.* **86**, 219 (1954)) and Adlersberg, L. E. Schaefer and Wang (*Science* **120**, 319 (1954)) have found that, in spite of increased levels of serum lipids, cortisone given to cholesterol-fed rabbits retarded atheroma formation. The experiments of J. Seifter *et al.* (*Proc. Soc. Exp. Biol. Med.* **83**, 468 (1953)) and of Adlersberg, Wang and Strauss (*J. Mt. Sinai Hosp.* **24**, 655 (1957)) demonstrated that hyaluronidase counteracts the effect of cortisone. Accordingly, the pathogenesis of atherosclerosis may be related to changes in mucopolysaccharides.

In order to determine the degree and time of change in acid mucopolysaccharide concentration in the aorta developing atheromatosis, A. J. Bollet, Wang and Adlersberg (*Circulation Research* **8**, 88 (1960)) employed a chemical method recently developed (Bollet, *J. Clin. Invest.* **37**, 1013 (1958)).

Rabbits were fed a chow diet either with or without an addition of 1 g. cholesterol

per day. Animals were sacrificed at the beginning of the experiment and after one, two, three, six and seven months. At two-week intervals the following lipid fractions were determined: total cholesterol, ester cholesterol, phospholipid, total lipid and neutral fat.

The extent of atheroma formation was estimated and a small segment was taken for histological examination including hematoxylin and eosin, periodic acid Schiff and a modified colloidal iron stain. The remainder was used for chemical analysis of the acid mucopolysaccharide content.

Animals fed cholesterol for one month or longer had marked increases in serum cholesterol, phospholipids and total lipid values. The degree of atheroma formation in the aorta increased with time. Animals fed for only one or two months showed early pathological changes, and animals fed for three months or longer showed moderate to severe lesions. Tissues taken from control animals and stained with colloidal iron stain gave only traces of color. Animals fed cholesterol for two months, however, showed a marked increase in staining, although the atheromatosis was only 1 plus. In aortas classed as 3 or 4 plus atheromatosis, the colloidal iron staining was distinctly less.

Chemical determination of the acid mucopolysaccharide concentration in the aorta was done by two methods involving analysis for uronic acid, the carbazole method and the orcinol method. Animals fed the ration

with cholesterol for one month showed no change from the control value. At two months, the values were lower than normal in some cases but returned to normal or control levels after three months of feeding. Significant elevation did not occur except in animals fed for six months or longer. The carbazole method gave higher values and showed greater increased uronic acid values with time.

Increases in acid mucopolysaccharide concentration occurred in a few of the animals with moderate atheromatosis and in all but one animal with severe atheromatosis. Animals with 3 plus atheromatosis averaged 517 mg. per cent acid mucopolysaccharide. This value was significantly different from the value in control animals with a *p* value of less than 0.001. Comparison of acid mucopolysaccharide concentrations in control animals with animals having less than 3 plus

atherosclerosis showed no significant difference.

In contrast to the chemical findings, histochemical observations using a modified colloidal iron stain showed an increased staining early in atheroma formation. The changes did not become more marked as atheromatosis became more advanced. This result indicates a need for study of the specificity of colloidal iron staining for acid mucopolysaccharides as well as information about factors influencing the affinity of tissue for this stain.

The chemical determinations indicate that an increased concentration of acid mucopolysaccharide is secondary to the deposition of lipid. Nevertheless, the results do not rule out the possibility that slight changes in acid mucopolysaccharide concentrations in the intima, undetectable by chemical techniques, may precede the deposition of lipid in the aorta.

### SERUM PROTEINS IN VITAMIN E DEFICIENCY

*With the onset of exudates in vitamin E-deficient chicks, the albumin to globulin ratio in the serum is decreased. However, the decrease in itself is not believed to be the cause of exudative diathesis.*

Chicks fed diets deficient in vitamin E present a syndrome characterized by subcutaneous hemorrhagic edema over the breast. This exudative diathesis can be prevented and cured by vitamin E and also by trace amounts of selenium (E. L. Patterson, R. Milstrey and E. L. R. Stokstad, *Proc. Soc. Exp. Biol. Med.* **95**, 617 (1957); K. Schwarz *et al.*, *Ibid.* **95**, 621 (1957)). Originally, H. Dam and J. Glavind (*Naturwissenschaften* **28**, 207 (1940)) concluded that capillary damage was the initial lesion involved, but the recent findings of J. Goldstein and M. L. Scott (*J. Nutrition*, **60**, 349 (1956)), showing that the serum proteins of vitamin E-deficient chicks are altered, have raised the question as to whether osmotic changes in the vascular system may not be responsible for the condition. B. G. Creech

*et al.* (*Ibid.* **64**, 55 (1958)) have shown that changes in the serum protein pattern precede the onset of exudative diathesis.

J. G. Bieri and C. J. Pollard (*J. Nutrition* **69**, 301 (1959)) have been particularly disturbed by the fact that no clear sequence of the events observed separately by different investigators had been established. Because the rate of depletion of vitamin E and hence the rate of appearance of symptoms varies in any group of chicks, they were of the opinion that a definite picture could only be obtained by following the progress of the deficiency in individual chicks. They, therefore, undertook such a study using paper electrophoresis as the criterion of changes in serum proteins of New Hampshire chicks fed vitamin E-deficient and control diets.

The vitamin E-free diet contained in per cent: soybean protein, 30; torula yeast, 15; stripped lard, 4; salt mixture, 6; vitamin mix, 0.2; L-cystine, 0.3; DL-methionine, 0.2; glucose, 44.3. On this diet, the authors found exudative diathesis appearing in 80 per cent of the birds within 14 to 25 days, at which time weights were from 125 to 300 g. Analysis of serums from nine control chicks, ranging from 14 to 21 days of age and fed on four different control diets, gave an average total serum protein of  $3.52 \pm 0.7$  per cent and an average albumin to globulin ratio of  $0.425 \pm 0.16$ . W. E. Vanstone *et al.* (*Canad. J. Biochem. Physiol.* **33**, 891 (1955)) have reported similar serum protein findings.

To observe the serum proteins prior to development of exudative diathesis, chicks fed the vitamin E-deficient diet were bled at intervals beginning on the tenth day following the start of the diet. Serial samples were obtained in this manner on six chicks.

Two to three days prior to the appearance of external symptoms, total serum proteins and the albumin to globulin ratios were found to be within the normal range. As soon as exudates were noticed, the albumin to globulin ratios had fallen considerably, but the decrease of total serum proteins was only slight for four chicks, with a significant decrease for one and a slight increase for another. In two chicks that had been checked 24 and 48 hours prior to symptoms, there was evidence that the decrease in albumin to globulin ratio began more than 24 hours before the edema occurred. The only obvious change in three of the serums was an increase in the alpha-2- and alpha-3-globulin areas.

Twelve chicks were bled two to eight days following spontaneous recovery from exudative diathesis. In three of the chicks, the normal pattern had been re-established. However, in the other nine birds there was a marked increase in the alpha-2-globulin peak as well as in the alpha-3-globulin peak and the beta- and gamma-globulin peaks. Albumin was considerably decreased. The change in the alpha-2-globulin was the most

consistent feature of these serums. The total protein in the serum was usually elevated above the normal range. The albumin to globulin ratios varied from near normal to very low levels, and occasionally chicks with very low ratios would become moribund after apparently recovering, cease to eat, and die within a week. After three or four weeks on the same deficient diet, most chicks that had recovered spontaneously showed essentially normal serum protein patterns. Administration of vitamin E or selenium restored the normal pattern to afflicted chicks in approximately one week.

The authors have considered the status of the proteins throughout the various stages of the condition and have reached the following conclusions: (1) Inasmuch as the albumin to globulin ratios during the recovery phase were often lower than when exudates were present, it is evident that the decreased albumin concentration alone could not account for the edema. (2) It would not appear that a decrease in total serum proteins could be involved, since some chicks with the exudative condition had higher serum protein levels than chicks without symptoms. (3) Since these observations seem to rule out osmotic changes as being of primary importance in the development of exudative diathesis, the original hypothesis of Dam and Glavind (*loc. cit.*) that capillary damage is the initial lesion appears to remain as the most logical explanation.

Bieri and Pollard further point out that the condition of exudative diathesis is probably caused by dietary conditions leading to the formation of peroxides in the tissues. The most important elements of the diet in this respect are unsaturated fat or certain mineral combinations which promote peroxidation of the body lipids (Bieri *et al.*, *Proc. Soc. Exp. Biol. Med.* **99**, 262 (1958)). Either vitamin E or selenium can prevent this, but, although the antioxidant nature of alphatocopherol is well known, no similar function for selenium has been described. The authors point out that if the damage to capillaries



is caused by products of autoxidation of fatty acids, then it appears necessary to propose a role for selenium in the sequence of reactions which constitute autoxidation.

The re-establishment of normal serum protein patterns in vitamin E- and selenium-deficient chicks would appear to rule out a

direct function for these metabolites in protein synthesis. It has also been shown (Pollard and Bieri, *Biochem. Biophys. Acta.* **34**, 420 (1959)) that the activity of certain respiratory enzymes is similar in vitamin E-deficient and control chicks as long as ten months after introduction to the diet.

## PANTOTHENIC ACID AND ADRENOCORTICAL FUNCTION

*Administration of the antivitamin, omega-methyl-pantothenic acid, to rats resulted in impairment of production of corticosterone by the adrenal glands.*

Pantothenic acid, a component of co-enzyme A, has been found to be essential for many species of animals (N. O. Kaplan and F. Lipmann, *J. Biol. Chem.* **174**, 37 (1948)). Without it, rats fail to grow and develop achromotrichia (graying of the hair). With severe degrees of pantothenic acid deficiency, adrenal insufficiency, adrenal atrophy, necrosis and hemorrhage may occur. F. S. Daft demonstrated that pantothenic acid could prevent this adrenal necrosis and hemorrhage, and others have confirmed his work (Daft, W. H. Sebrell, S. H. Babcock, Jr. and J. H. Jukes (*Pub. Health Reports* **55**, 1333 (1940)). Microscopically, the adrenals of deficient animals show hyperemia with areas of hemorrhage and disruption of the normal cortical architecture, particularly of the zona fasciculata and the zona reticularis (L. L. Ashburn, *Ibid.* **55**, 1337 (1940)).

Special techniques have demonstrated that ketosteroids disappear first from the two inner zones of the cortex (reticularis and fasciculata) with a general loss of fat droplets in the same area. Shortly thereafter necrosis and hemorrhage appear. In severe cases, the total cortex is destroyed except for a thin layer of the zona glomerulosa (H. W. Deane and J. M. McKibbin, *Endocrinology* **38**, 385 (1946)). It has been found that administration of adrenocortical extracts will prevent changes in the color of the fur and will prevent the undue susceptibility which these animals may have to

water intoxication (R. Gaunt, M. Liling and G. W. Mushett, *Ibid.* **38**, 127 (1946)).

Some investigators have considered the possibility that the adrenal cortex becomes hyperstimulated, due to the stress of this vitamin deficiency and that the changes represent a stage of exhaustion. Apparently deficient rats exhaust their supplies of adrenal cholesterol and cannot replenish it adequately (M. E. Dumm, H. Gershberg, E. M. Beck and E. P. Ralli, *Proc. Soc. Exp. Biol. Med.* **82**, 659 (1953)). Although lipogenesis itself does not seem to be altered in deficient animals, the depletion of cholesterol by the adrenal has been found repeatedly (K. Guggenheim and R. E. Olson, *J. Nutrition* **48**, 345 (1952)).

While a deficiency of pantothenic acid can be induced in many animals by elimination of this vitamin from the diet, it can be induced more easily and more quickly by the addition of an antagonist, omega-methyl-pantothenic acid. However, there is reason to believe that this antagonist can, in certain instances, function as the active vitamin in some respects while inhibiting the natural vitamin effect in others (D. W. Woolley, *A Study of Antimetabolites*, p. 79. John Wiley & Sons, New York (1952)).

In an effort to determine whether omega-methyl-pantothenic acid might be a useful therapeutic agent to induce adrenal insufficiency for treatment of such conditions as neoplastic disease, A. D. Goodman (*Endocrinology* **66**, 420 (1960)) investigated the

effects of this antagonist upon the adrenal function of rats. He compared the effects of this antivitamin with those of the vitamin deficiency itself with respect to adrenal function and histologic appearance in order to determine the combined effect of the antagonist and adrenocorticotrophic hormone (ACTH), and to learn whether the administration of an anticoagulant to deficient animals would result in greater degrees of adrenocortical hemorrhage which, in effect, would be the equivalent of adrenalectomy.

Male rats were fed a basic diet which was adequate in all respects except for its lack of pantothenic acid. This diet was used in all the following studies. Also, in each group, control animals were pair-fed to assure an equal intake of foods by the experimental and normal animals. In the first study, one group of animals was made deficient by simple deprivation while their controls were given the active vitamin. In a second study, omega-methyl-pantothenic acid was given in a quantity of 500 mg. per 100 g. daily, while their controls received 0.3 mg. per 100 g. In a third study, a group of animals was given 750 mg. of the antagonist per 100 g. daily and the control animals were maintained on the deficient diet. About the eleventh day, the adrenal veins of half of the animals in each group were cannulated and blood collected, while the remaining animals were given an injection of 65 mg. of the active vitamin subcutaneously and adrenal venous blood was collected 24 hours later.

In a further study, a group of animals was given the antagonist for 11 days, after which time ACTH dissolved in saline was given to half of them subcutaneously in a dose of two units four times daily, plus an additional two units 20 minutes before the collection of adrenal venous blood. A second group received subcutaneously four units of ACTH in beeswax and peanut oil suspension daily for five days before the operation, and an additional four units in saline 20 minutes before collection of adrenal venous blood.

In the final experiment, a group of rats was given the antagonist for 11 days while their controls were given none. Four to five days before sacrifice, 0.2 mg. of sodium warfarin was injected intraperitoneally each day. Hemorrhages were noted at the sites of administration. On the day of sacrifice, large doses of active vitamin K were given.

In each study, blood was collected for 12.5 minutes from the left adrenal veins during ether anesthesia. Following this, the animal was killed and the right adrenal was saved for histologic examination while the left was weighed. The concentration of corticosterone in adrenal venous plasma was measured by a modification of the method of R. E. Peterson (*J. Biol. Chem.* **225**, 25 (1957)).

Deficient rats secreted 47 per cent less corticosterone than did their normal controls, and their adrenals appeared gray-purple in color as compared with light pink of the normals. Microscopic examination disclosed adrenal necrosis in only four of the fourteen deficient animals, and in these four the zona fasciculata and zona reticularis showed almost complete absence of normal vasculization of the cytoplasm.

The antagonist, when given alone for 18 days, resulted in a 39 per cent decrease in secretion of corticosterone. Rats receiving both the deficient diet and the antagonist secreted 46 per cent less steroid than did their controls. However, injection of the active vitamin 24 hours before collection of blood reversed this abnormality almost completely. Rats which had received the antagonist had gray-purple adrenals, but there was no hemorrhagic necrosis. Histological examination revealed loss of cytoplasmic vasculization.

The administration of ACTH to rats treated with the antagonist disclosed that these animals were capable of responding to the trophic hormones. No evidence of adrenal necrosis was found. Administration of the anticoagulant, sodium warfarin, to animals receiving the antagonist did not induce gross or histologic evidences of changes.

These results confirm other findings that pantothenic acid deficiency in the rat reduces adrenal secretion of corticosterone (B. B. Longwell, A. E. Reif and E. Hansbury, *Endocrinology* **62**, 565 (1958)). It is evident that the antagonist, omega-methyl-pantothenic acid, produced identical changes in these animals. The complete reversibility following administration of pantothenic acid indicates that this was a specific response. Since pantothenic acid is an integral part of coenzyme A, which in turn is a factor necessary for the synthesis of steroids from acetate (H. P. Klein and F. Lipmann, *J. Biol. Chem.* **203**, 101 (1953)), it was assumed by the author that this was the mechanism for impairment of adrenal function. Failure of the antagonist to induce adrenal hemorrhage and necrosis could have been due to brief administration, inadequate dose of the antagonist, or to a different effect than that produced by simple deficiency.

The theory that adrenal hemorrhage results from over-stimulation, by trophic hormones, of a gland which is functioning inadequately was not supported by the finding of Goodman that ACTH was capable of stimulating the adrenal glands of animals depressed by the antagonist. He considered several possibilities: (1) that ACTH might contain sufficient quantities of pantothenic acid to block the antagonist; (2) that under intense ACTH stimulation, synthesis of coenzyme A might be accomplished by an alternate metabolic pathway, or (3) that partial insufficiency of endogenous produc-

tion of ACTH may be part of the mechanism whereby the antagonist exerts impairment of adrenal function. This latter was considered unlikely.

No explanation was offered for the failure of anticoagulants to induce adrenal hemorrhage, but neither was this demonstrated in simple pantothenic acid deficiency.

In human pantothenic acid deficiency, an illness can be produced by elimination of the vitamin from the diet or by the administration of large quantities of omega-methyl-pantothenic acid (W. B. Bean, R. E. Hodges and K. Daum, *J. Clin. Invest.* **34**, 1073 (1955); Hodges, Bean, M. A. Ohlson and R. Bleiler, *Ibid.* **38**, 1421 (1959)). Although initial studies with human subjects seemed to indicate findings compatible with adrenocortical malfunction, further investigation failed to confirm this. It seems possible that human beings react in a somewhat different manner to a deficiency of this vitamin than do animals, or that a more severe degree of deficiency might result in failure of the adrenal cortex. However, such drastic investigation would not be justified in man.

Goodman has demonstrated that both the spontaneous deficiency and that produced by an antagonist result in impairment of adrenocortical function. His idea of inducing adrenal hemorrhage by means of employing an anticoagulant simultaneously should be explored further in deficient animals. Conceivably, this could become an important therapeutic method for treating patients now subjected to surgical adrenalectomy.

### LIPID MOBILIZING HORMONE

*Some evidence exists that a lipid mobilizing hormone is elaborated by the posterior lobe of the pituitary. Partially depolymerized hyaluronic acid blocks this effect.*

The intricate mechanisms whereby the body regulates lipid metabolism continue to puzzle the imagination. The following is a unique concept.

In a summary of the past six years of studies, C. J. D. Zarafonitis, J. Seifter, D. H. Baeder and J. P. Kalas (*A. M. A. Arch*

*Int. Med.* **104**, 974 (1960)) describe a factor which releases fats into the circulating blood. They found that hyaluronidase and partially depolymerized hyaluronic acid induce clearing of lipemic serum of animals and that cortisone inhibits this effect. Studies of the nephrotic syndrome disclosed the presence of

a substance which the authors called "lipid mobilizer hormone" (LMH), which was prepared from the serum of horses previously treated with cortisone. This substance, which was dialyzable, and seemed to be an octapeptide, was found to release triglycerides from omental and mesenteric fatty stores (Seifter and Bader, *Proc. Soc. Exp. Biol. Med.* **86**, 709 (1954); **91**, 42 (1956); **95**, 318, 469 (1957)).

Single injections of LMH into fasting patients induced increases in concentrations of cholesterol, fatty acids and lipid phosphorus of the peripheral blood. Patients fed a diet low in fat and given daily injections of LMH for two weeks developed marked degrees of hyperlipemia (Zarafonitis *et al.*, *Am. J. Med. Sci.* **234**, 493 (1957)). Pre-feeding patients with glucose or fats prevented this hyperlipemic response, but pre-feeding with amino acids did not. In animals, depletion of hepatic glycogen or exposure to substances injurious to the liver predisposed them to an exaggerated response to LMH. Evidence of endogenous secretion of LMH was obtained by studying patients during and after operation and animals subjected to various forms of stress. In these instances, a great increase in the concentration of venous fatty acids occurred, but the arterial concentrations did not increase. Cholesterol was affected to a lesser extent.

The posterior lobe of the pituitary gland of animals contained significant amounts of LMH and hypophysectomy prevented the lipemic response to stress. Accordingly, the authors proposed the theory that stress stimulates release of pituitary adrenocorticotrophic hormone, which in turn releases adrenal cortical steroids. These steroids then stimulate the posterior pituitary to release LMH, which acts on omental and mesenteric fat beds to release neutral fats into the portal circulation.

The authors studied partially depolymerized hyaluronic acid and found that it prevented the lipemic response of both animals and man to LMH. It also prevented

the hyperlipemic response to stress but not the release of LMH into the blood, the effect of which was apparently blocked. Accordingly, the authors gave partially depolymerized hyaluronic acid to two subjects with familial hyperlipemia and found that a dose of 200 mg. three times daily before meals resulted in a marked decrease in their serum concentrations of fatty acids and of cholesterol.

Many endocrine secretions are known to alter lipid metabolism. Thus, in patients with untreated diabetes mellitus, hepatic glycogen becomes depleted, glucose utilization is inhibited and lipogenesis is reduced sharply. Fat is mobilized and is catabolized at an accelerated rate. Presumably this accounts for the ketosis which is a familiar complication (F. L. Engel, *A. M. A. Arch. Int. Med.* **100**, 18 (1957)). E. H. Strisower *et al.* (*J. Clin. Endocrinol. Metab.* **18**, 1418 (1958)) demonstrated that diabetic patients given inadequate amounts of insulin developed hyperlipemia, which was corrected by administering more insulin.

Both epinephrine and nor-epinephrine may cause hyperlipemia, provided adrenal cortical steroids are present in normal amounts. Excessive amounts of cortisone given to intact animals will exaggerate the lipemic response to adrenal medullary hormone (M. C. Schotz and I. H. Page, *Proc. Soc. Exp. Biol. Med.* **101**, 624 (1959); E. Shafrir and D. Steinberg, *J. Clin. Invest.* **39**, 310 (1960)).

The thyroid gland also is involved in lipid regulation. In a study of hyperthyroid patients, C. Rich, E. L. Bierman and I. L. Schwartz (*J. Clin. Invest.* **38**, 275 (1959)) found that serum concentrations of non-esterified fatty acids were increased when a person's metabolic rate was increased, and these concentrations served as a rough index of the rate of mobilization of fat. Euthyroid subjects showed an increase in these fatty acid concentrations within six hours after the administration of L-triiodothyronine. Growth hormone also has been shown to in-

crease the serum concentration of non-esterified fatty acids in both animals and man. However, pre-feeding with glucose or with food prevented this effect (M. S. Raben and C. H. Hollenberg, *Ibid.* **38**, 484 (1959)).

These interrelationships make the simplified theory of Zarafonetis *et al.* seem implausible. It is possible that other endocrine hormones could exert their effect through the pituitary via this hypothetical lipid mobilizing hormone. Nevertheless, such a theory needs to be tested and confirmed by others before it can be accepted with finality. The experimental data is meager and biological variations are great.

The studies of partially depolymerized hyaluronic acid are intriguing. A similar study (P. Constantinides *et al.*, *Brit. Med. J.* **1**, 535 (1960)) disclosed that sulfated polymannuronides had a comparable effect, in that they could activate lipoprotein lipases and stimulate removal of lipids by the reticulo endothelial system. (Heparin apparently acts by the former mechanism only.)

The work of Zarafonetis *et al.*, although lacking in conclusive evidence, is imaginative and should stimulate efforts to confirm or deny the existence of a lipid mobilizing hormone.

### SEVERE UNDERNUTRITION IN ANIMALS

*Severe restriction of food intake in poultry and pigs for many months produces a variety of changes. Both species when starved are highly susceptible to cold and infection, the primary causes of death.*

The name of C. M. McCay is frequently thought of when one considers experimental undernutrition, especially as it influences longevity (*J. Nutrition* **10**, 63 (1935)). He and his co-workers showed that when weanling rats were kept from gaining weight for periods of a year or more and then full fed, they lived much longer than the controls which had been full fed from the beginning. Protracted undernutrition in these rats postponed the attainment of maturity. This might be considered the explanation for the prolongation of the lives of the above undernourished animals.

Although many studies were carried out on semi-starved human beings during and after World War II, there has been relatively little experimental work with animals since that time. However, a paper by R. A. McCance suggests a resurgence of work in this field (*Brit. J. Nutrition* **14**, 59 (1960)).

To a certain extent, McCance repeated the work of McCay, but with poultry and pigs. Day-old cockerels were fed a commercial chick food until they weighed about 100 g. At this time they were divided into two

groups. One-half the chicks were given the diet and water on an ad libitum basis, while the other chicks were fed just enough to maintain their body weights. The latter birds were fed individually in a large box partitioned so that a number of birds could be fed at one time and were provided with an extra source of heat throughout the experimental period. These birds received from 8 to 10 g. of food per day, while the full-fed chicks ate about 60 g. per day.

The semi-starved birds showed only a slight increase in body weight over a period of six months and lost most of their feathers, becoming almost naked. After a time they developed a few weak wing feathers and some longer ones on the tail. Their claws continued to grow. Their skin, however, showed no marked sign of abnormality. Their beaks increased in size and occasionally the upper half of the beak grew longer than the lower half.

The semi-starved chicks spent a great deal of time picking at the empty food trays. However, this was partly caused by a luminous lamp used for heating. When this was replaced

with an infrared lamp, the birds were quiet. There was no sign of nutritional edema in any of the birds.

The pigs in the undernourished group were removed from the sow and the commercial ration for a progressively longer time each day toward the end of lactation. After weaning, they were fed enough of the commercial ration to maintain their weight (7 to 15 kg.) for periods as long as 15 months.

One problem was the high mortality of the undernourished pigs resulting from parasitic and infectious epidemics. The semi-starved pigs died in the early stages of the study with greatly distended abdomens. The intestines of some of these animals were filled with mature and fully-grown worms. The worms, however, could be eliminated by treatment with a vermifuge (piperazine adipate). Gastrointestinal infections killed 50 per cent of the starved animals in one experimental group. Another large group died from general infections. The matted hair and the encrusted skin of the starved pigs produced a great deal of scratching which frequently led to an abrasion of the skin followed by an infection in various parts of the body. The rapidly growing animals, on the other hand, showed no problem on either of these scores.

Another cause of the high mortality of the undernourished pigs was their susceptibility to cold. Some of these animals died away from the source of heat used to keep their pens warm.

The starved pigs gave the impression of wizened age; they had lost much of their hair and the skin of the back and legs was frequently crusted over with a dry keratinous material. Hyperkeratosis was a prominent finding. Histological examination showed considerable abnormality in the cell structure of the epidermis. Even after one or two years of undernutrition, however, the skin changes were reversible with ad libitum feeding.

The skin changes were not due to a vitamin A deficiency. The livers of the full-fed pigs contained 65 to 130 I.U. of vitamin A

per gram, whereas the livers of the semi-starved pigs had 100 to 800 I.U. per gram.

There were prominent changes in the gait and the stance of the starved pigs. They stood and walked "on their heels." Their nails continued to grow in spite of the fact that other growth processes were at a standstill.

The voices of the starved pigs changed from "the usual grunt to something more like a croak."

During the early part of the starvation experiment, the pigs became excited at feeding time and occasionally suckled each other at the tail or penis. When there was nothing to disturb them, they lay quietly in their insulated cages and did not display the activity which has been reported as a characteristic feature of undernourished rats.

Edema was seen in only one or two of the pigs, but the others never showed any signs of pitting edema.

Many of the organs of the undernourished pigs were of normal size, when expressed as a percentage of the body weight. On this basis, the spleens were approximately one-fourth the size of those seen in the normal controls. The opposite was true, however, of the adrenal glands; in seven undernourished pigs they represented 0.052 per cent of the body weight versus 0.019 per cent in five controls.

The starved pigs had a hemoglobin level of 10.2 g. per 100 ml., whereas the controls of the same age had 15.4 g. The white cells in the starved pigs averaged  $7.7 \times 10^3$  per cubic millimeter, while in the full-fed animals of the same age, it was  $12.0 \times 10^3$ . The hematocrit in the starved pigs was approximately one-half that of the full-fed pigs.

Blood and urine urea nitrogen levels of the starved pigs were normal and, although no specific tests of kidney function were carried out, McCance stated that "there is no reason to think that the kidneys of these animals have ever been functionally abnormal."

The growth of the various bones of the semi-starved pigs was not inhibited to the



same extent. The ribs, for instance, continued to grow somewhat in length so that the starved pig had a large thoracic cage. The vertebrae were reported as continuing their growth, but no quantitative data were presented. The molars of the pigs continued to appear, even though there was no room for

them in the mouth. X-ray examination showed the molars to be impacted.

These studies are of interest in that they serve as the initial report of what may well be some important observations on the influence of undernutrition in the pig, a valuable experimental animal for such study.

### GALLSTONE FORMATION IN THE HAMSTER

*Hamsters fed a non-lithogenic diet for 77 days following a 63-day period on a lithogenic diet, had fewer gallstones with lower cholesterol content than at the end of the 63-day period.*

The experimental production by dietary means of stones in the gallbladder of hamsters has been described earlier by F. Christensen, H. Dam and G. Kristensen (*Acta. Physiol. Scandinav.* **36**, 329 (1956)). The stones formed were either cholesterol or amorphous pigment particles, and were consistently induced by feeding the animals a diet made up of 20 per cent crude casein, 72.3 per cent sucrose, 0.2 per cent choline chloride and 2 per cent lard containing vitamins A and D, plus salt mixture and vitamins. All of the ten animals maintained on this diet for 63 days developed gallstones. After washing and drying, the combined weight of the stones was 9.39 mg.

When a second group of hamsters was kept on the same diet for 140 days, 11 of the 12 animals were found to have gallstones. The combined weight of these stones was 10.47 mg., indicating that there was not much increase in growth during the latter 77 days on the lithogenic diet.

A third group of hamsters was kept on the lithogenic diet for 63 days and then transferred to a non-lithogenic diet for 77 days. This latter diet consisted of 20 per cent crude casein, 36 per cent yeast, 28.3 per cent polished white rice, salt mixture, vitamins and choline chloride. The fat content of this diet was increased to 10 per cent, incorporating vitamins A and D. Also 0.13 per cent copper sulfate was added to the salt mixture. Of the ten hamsters autopsied, only five had

gallstones while the total weight of the stones removed was only 2.65 mg. Since the first group of hamsters had been maintained for the same number of days on the lithogenic diet but had considerably more gallstones than group three, the authors assumed that the non-lithogenic diet actually caused a dissolution of the gallstones already formed. The average cholesterol content of the gallstones from animals on the stone-producing diet was about 55 per cent, while the cholesterol content of stones from animals on the non-lithogenic diet was found to be only 0.7 per cent.

These investigators believe that in the hamster the oversaturation of the bile-mucin with cholesterol leads to the precipitation of a cholesterol-type stone, although there was no discussion as to why these dietary changes lead to the formation of cholesterol stones. The possibility exists that the lithogenic diet, being low in fat, may be responsible for a decreased contraction of the gallbladder, and this in turn may be an important factor in the stone formation.

More recently, W. Van der Linden, F. Christensen and H. Dam (*Acta. Chir. Scandinav.* **118**, 113 (1960)) have studied the formation of gallstones in hamsters on a lithogenic diet before and after the removal of the gallbladder. The animals maintained on the lithogenic diet developed a large number of stones. However, those that had the gall-

bladder removed did not develop stones in the bile duct. Therefore, these investigators believe that the stones are formed in the gallbladder and then may migrate to either the common bile duct or the cystic duct.

Thus these investigators have established a methodology for the experimental pro-

duction of cholesterol stones in the gallbladder of hamsters. By adjusting the diet it is possible to get stone formations in almost all of the animals studied. Investigations of this type may lead to more basic studies of both the rate of formation and the dissolution of such stones.

### METHIONINE IN METABOLISM

*Iodocasein causes decreases in chick weight gain, enlarged livers and increased oxygen uptake. Methionine produces opposite effects and, in certain growth phases, counteracts the effect of iodocasein.*

From the extensive studies of metabolic roles of amino acids now being conducted, the conclusion emerges that a former view of amino acids purely as substrates for protein synthesis must be enlarged, at least in the case of several amino acids, to include other functions. One such amino acid appears to be methionine.

For several years, L. W. Charkey has been attempting to elucidate the role of vitamin B<sub>12</sub> in amino acid metabolism as affected by stresses applied to experimental chicks (Charkey *et al.*, *Proc. Soc. Exp. Biol. Med.* **73**, 21 (1950); *J. Biol. Chem.* **210**, 627 (1954); *Poultry Sci.* **32**, 630 (1953); **38**, 899 (1959)). One such stress factor is the thyroid hormone, or ultimately, dietary sources of it, such as iodinated casein. In some preliminary experiments it was noted that vitamin B<sub>12</sub> reversed some of the effects of iodocasein, notably in preventing development of oversize livers. However, of greater interest was the finding that methionine, unlike vitamin B<sub>12</sub>, reversed the capacity of iodocasein to increase the oxygen uptake of chicks. The details of these studies have been confirmed in the following carefully controlled experiments (Charkey, *J. Nutrition* **69**, 295 (1959)).

Animals used were single-comb white leghorn cockerels. There were 20 chicks per group in the first experiment and 18 per group in the second. The basal diet consisted, in per cent, of yellow cornmeal, 66.0; soybean meal (50 per cent protein), 13.5; dehydrated alfalfa meal (17 per cent protein),

5.88; dried brewers' yeast, 5.0; gelatin, 4.0; steamed bone meal, 1.7; limestone, 1.0; DL-phenylalanine, 0.214; DL-tryptophan, 0.041; iodized salt, 0.5; potassium chloride, 0.2; and magnesium sulfate, 0.242. Vitamins and trace minerals in milligrams per kilogram of diet were as follows: manganese sulfate, 50; iron sulfate, 20; copper sulfate, 2.0; zinc chloride, 0.2; pyridoxine hydrochloride, 2.5; folic acid, 0.5; biotin, 0.1. Calculations indicated that this diet contained 19.7 per cent protein, and all essential amino acids, with the exception of methionine, were present at greater than National Research Council requirement levels for a 20 per cent protein diet for chicks. Methionine, after correction for the simultaneous cystine deficiency, was present at only 36 per cent of the requirement level.

The dietary variables in a 2<sup>3</sup> factorial design were vitamin B<sub>12</sub> at 25 micrograms per kilogram of diet, iodocasein at 0.05 per cent and DL-methionine at 0.362 per cent of the diet. This was the amount calculated to bring the total methionine to the requirement level in supplemented groups.

Amounts of free methionine, histidine and isoleucine in blood, liver and excreta were measured, as were oxygen uptakes of intact birds, body weight gains, feed utilization efficiencies, weights of excreta, alcohol-insoluble nitrogen and uric acid contents of excreta and liver as percentages of body weight.

All supplements, including methionine,

produced increases of the order of 5 to 10 per cent in oxygen uptake at eight to ten days of age. However, after two weeks of age, vitamin B<sub>12</sub> failed to produce any significant effect on oxygen uptake, and methionine actually decreased it. At four weeks of age, the methionine effect was lost in the presence of iodocasein and was of doubtful significance in its absence. Thus it appears possible that the methionine effect of decreasing the oxygen consumption occurs only between two and four weeks of age in leghorn chicks. The iodocasein effect seems to be of longer duration, but possibly could be counteracted by higher levels of methionine.

In both experiments, body weight gain and feed utilization efficiency were inversely related to the oxygen uptakes. Liver weight as a percentage of body weight was increased in proportion to the oxygen uptake.

One purpose of the experiment was to determine whether vitamin B<sub>12</sub> would enhance conversion of an amino acid in excess (histidine or isoleucine) to one in deficiency (methionine). This did not take place. Except for an increase in methionine of the blood and excreta as a result of supplementation with this amino acid, free amino acid

levels showed no relationship to dietary supplements.

Weights of excreta were roughly parallel to feed consumed and growth produced. No relationship to any of the dietary supplements was found. Total nitrogen and uric acid values in the alcohol-extracted excreta tended to be inversely related to methionine supplementation and to growth obtained. The data may indicate a specific relationship to vitamin B<sub>12</sub> (which vitamin is known to play a role in purine biosynthesis) since uric acid excretion seemed slightly reduced by vitamin B<sub>12</sub> supplementation.

Since thyroid hormone is a metabolic uncoupler of oxidative phosphorylation, it is suggested by the author that methionine functions in metabolism as a coupling agent or by enhancing biosynthesis of such an agent or of interfering otherwise with the functioning of the thyroid hormone as an uncoupler. He further speculates that methionine may be a factor in linking anabolic reactions to oxidative reactions, thereby making available the energy provided by the latter for more effective metabolic function. Herein lies a possible basic explanation for some of the results of its deficiency.

## DIETARY FAT AND SERUM CHOLESTEROL OF CHICKS

*Serum cholesterol levels in chicks are raised by feeding saturated acids and lowered by polyunsaturated and possibly monounsaturated acids. They appear to be inversely correlated to iodine number of the fat.*

Since the initial reports that the character of the dietary fat might be related to the level of blood cholesterol, this problem has been the subject of intensive research in many laboratories. Although there has been general agreement that some such relationship exists, its exact nature is still obscure. For example, it is still uncertain whether the blood cholesterol of man and experimental animals is lowered by inclusion in the diet of fats with high iodine numbers, high content of polyunsaturated acids, high content of essential fatty acids, plant sterols or, possibly,

merely a low content of saturated acids (*Nutrition Reviews* 18, 21 (1960); 17, 10 (1959); 16, 68, 83, 131 (1958); 15, 1, 39 (1957); 14, 327 (1956)).

Recently, the studies of D. M. Hegsted and his co-workers have revealed other possible relationships. In experiments with rats fed cholesterol and cholic acid it was found that not the total unsaturation of the dietary fat but the product obtained by multiplying the linoleic and arachidonic acid content by that of the saturated fatty acids correlated best with the inverse of the serum cholesterol

concentration (Hegsted, S. B. Andrus, A. Gotsis and O. W. Portman, *J. Nutrition* **63**, 273 (1957); Hegsted, Gotsis and F. J. Stare, *Ibid.* **63**, 377 (1957); Hegsted, Gotsis, Stare and J. Worcester, *Am. J. Clin. Nutrition* **7**, 5 (1959)). Regression equations relating the serum cholesterol values with the relative amounts of saturated monounsaturated and polyunsaturated acids had negative coefficients for the first and last types and positive coefficients for the monounsaturated acids. There was thus some evidence that, at least in rats, the oleic acid content of the diet might be the blood cholesterol-raising factor.

The authors were well aware of the limitations of these experiments and suggested that many variables would have to be taken into consideration and correlated together with the serum cholesterol before there could be much extrapolation of these results to other animals and conditions.

In a start at such a study, Hegsted, Gotsis and Stare (*J. Nutrition* **70**, 119 (1960)) performed similar experiments starting with day-old cockerels. Groups of chicks were maintained for three weeks on diets consisting of 52.7 per cent sucrose, 18 per cent casein, 5 per cent salt mixture, 10 per cent gelatin, 3 per cent cellulose, 1 per cent calcium phosphate, 0.3 per cent cystine and choline and adequate amounts of the water- and fat-soluble vitamins. The fat content of the diet was fixed at 10 per cent and was varied to give different proportions of saturated, monounsaturated and polyunsaturated acids. Cholesterol was fed as 0.2, 0.4 or 0.8 per cent of the diet.

In the first experiment, blood cholesterol levels (taken at the second and third weeks) were highest for the highest cholesterol levels

and for coconut oil. Safflower oil gave the lowest values followed by a safflower-coconut mixture and triolein.

In further experiments, several saturated and unsaturated fats and oils were mixed to give different proportions of the three types of acids and were fed with 0.8 per cent cholesterol. The calculated regression and correlation coefficients for the three acid types in the dietary fats of these experiments suggest that the saturated acids tend to elevate the serum cholesterol in chicks while the polyunsaturated acids tend to lower it and the monounsaturated acid (oleic) has little effect. These results were similar to those obtained by A. Keys and co-workers in human studies (Keys, J. T. Anderson and F. Grande, *Circulation* **19**, 201 (1959)). When the serum cholesterol values were plotted against the iodine number of the dietary fats, a fairly good correlation was observed for most cases. Interesting exceptions were the coconut-corn oil and coconut-cottonseed oil mixtures, which were about as effective as the corn and cottonseed oils themselves in maintaining a low serum cholesterol.

The authors again recognize the limitations of their experiments. Three weeks was probably too short a time to expect to get stable cholesterol values. The fat content of the diet itself is of importance but was not considered in these studies. Moreover, the role of oleic acid was still not clear. However, a promising start of a relatively rapid assay of serum cholesterol-lowering value of various fats has been made.

If further experiments can confirm and amplify these observations and extend them to man they will become most valuable.

### COLLOID GOITER IN THE HAMSTER

*Colloid goiter in hamsters has been produced by an iodine-deficient regimen followed by iodine refeeding. The gland has a morphological picture similar to that found in the diffuse colloid goiter.*

In 1908 D. Marine and W. W. Williams (*Arch. Int. Med.* **1**, 349 (1908)) suggested

that goiter must be classed as a nutritional disorder. This view was based on morpho-

logic and chemical studies of various types of goiters occurring naturally in man and other animals, and on the changes caused by the administration of iodine to animals with hyperplastic or colloid type of goiters. For experimental studies, Marine depended on dogs in which endemic goiters had already developed. He postulated that the pathogenesis of the goiter occurs in the following fashion. Iodine deficiency causes the normal gland to become hyperplastic and this continues until iodine is administered; finally, a colloid goiter is formed.

In man, colloid goiters with varying degrees of nodular hyperplasia and scarring are found in areas where the disorder is endemic. Until recently, the experimental production in laboratory animals, of this morphological picture has not been successful. Most experimental attempts to produce goiter yielded glands which were characterized by varying degrees of hyperplasia but without the diffuse colloid changes found in the naturally occurring disease.

Since the pathogenesis of the colloid goiter, as postulated by Marine, needed laboratory confirmation and since the routine experimental production of such a disorder would provide a valuable means for study, R. H. Follis, Jr. (*Proc. Soc. Exp. Biol. Med.* 100, 203 (1959)) has been attempting to produce this syndrome in various types of experimental animals (rabbits, rats, guinea pigs, hamsters and monkeys). He found that hamsters weighing between 75 and 140 g. developed colloid goiters when kept on either of two iodine-deficient diets for more than 125 days.

The first diet consisted entirely of ground whole unenriched corn, while the second was made up of 76 per cent ground corn, 20 per cent wheat gluten, 2 per cent sodium chloride and 2 per cent calcium carbonate. To each kilogram of diet number two, 500 mg. of a complete vitamin mixture were added. Both diets were considered iodine-deficient since the first diet contained 1.0 microgram and the second diet 2.5 micrograms of iodine per 100 g. The second diet allowed better growth

of the animals and more extensive changes of the thyroid gland. Male and female hamsters were maintained on the latter diet up to 350 days.

The evaluation of the effects of these diets on the thyroid was made following microscopic examination of sections of the gland. After being on the iodine-deficient diets for one to two weeks, no thyroid enlargement was noted. Grossly, however, both lobes showed increased vascularity. By the end of the second week, it was noted on microscopic examination that the colloid disappeared from the follicles of the thyroid gland. At this time the vascularity of the gland increased and Follis stated that "sometimes follicles appear to be virtually floating in a pool of blood." After 125 days on the diets, the enlarged gland was made up of hyperplastic follicles surrounded by prominent blood channels and a few of the follicles contained eosinophilic colloid. The hyperplasia of the thyroid became more extensive in hamsters maintained on the iodine-deficient diets for 325 days and more follicles contained colloid. However, the number of follicles containing colloid never exceeded 10 per cent.

Reappearance of colloid in some of the follicles led Follis to hasten this effect by administering iodine to animals maintained on a deficient diet for 125 days. Two weeks of iodine refeeding (10 mg. of potassium iodide added to 100 g. of diet) caused the follicles to fill with colloid and the gland to become grossly and microscopically less vascular. The size of the follicles increased to three times normal. When animals that had been on a deficient diet for over 300 days were refed iodine, they acquired even larger colloid-lined follicles than those fed the deficient diets for a shorter period.

This laboratory study further substantiates the concept that the formation of a colloid goiter results from hyperplasia induced by iodine deficiency, then followed by iodine repletion. It also provides a routine method for producing colloid goiter in a common laboratory animal. The morpho-

logical picture is similar to the diffuse colloid goiter. This study will open new avenues to the investigation of many questions concerning the colloid goiter. For example, is there a point in the development of the colloid

goiter beyond which the hyperplastic gland can no longer revert to the appearance of normal tissue, or may alternating cycles of depletion and repletion lead to a nodular goiter?

### HYPEROXALURIA CAUSED BY PYRIDOXINE DEFICIENCY

*Kittens fed a diet deficient in pyridoxine develop striking hyperoxaluria and renal deposits of calcium oxalate, with scarring; the disease is similar to human "idiopathic" hyperoxaluria and oxalosis.*

Little is known of the mechanism of primary hyperoxaluria and oxalosis in man (*Nutrition Reviews* 16, 75 (1958); 17, 104 (1959); 18, 159 (1960)) and few cases have been reported. The production of a comparable disease in an experimental animal is reviewed below and should help to advance our knowledge of the human disease.

S. N. Gershoff, F. F. Faragalla, D. A. Nelson and S. B. Andrus have described "Vitamin B<sub>6</sub> deficiency and oxalate nephrocalcinosis in the cat" under this title (*Am. J. Med.* 27, 72 (1959)). They used three- to six-month-old kittens fed purified rations complete except for pyridoxine hydrochloride, which was fed at 0, 1 or 2 mg. per kg. of diet. There were four animals per group and the experiments lasted three to six and one-half months.

All four cats on the unsupplemented diet developed acute vitamin B<sub>6</sub> deficiency in two to two and one-half months, with "growth failure, emaciation, convulsions and anemia."

Large quantities of oxalate were found in the urine and kidneys of the deficient animals; oxalate increased tenfold in the urine and ten- to one hundredfold in the kidneys over the amounts found in cats receiving the highest levels of pyridoxine. The urinary inorganic sulfate was excreted in increasing amounts as the pyridoxine content of the diet was increased.

The kidneys contained many oxalate crystals, especially in the cortices, where the heaviest deposits were associated with many irregular flat depressed scars 2 to 4 milli-

meters in diameter and involving one quarter to three quarters of the kidney surface. These changes were seen in all of the four cats receiving no pyridoxine, in three of the four given 1 mg. of the vitamin per kg. of diet, and in none of the cats fed 2 mg. of the vitamin per kg. of diet.

The scars were pyramidal, the apices reaching the inner cortex or deeper, and consisted of atrophy and loss of tubules with condensation of the stroma. Tubular dilatation or epithelial hyperplasia were rare. The glomeruli were apparently quite normal in general.

The crystals were doubly refractive and occurred chiefly in the cortex and in distal collecting tubules, occasionally beneath the renal pelvic epithelium. Older scars had fewer crystals than the fresher ones. "The crystals occurred as needles and rods arranged in symmetrical rosettes or sheaves or as irregular plates and smaller fragments." They gave most of the reactions of calcium and of oxalate. In general, no crystals were found in other tissues.

Other pathologic lesions found in the deficient cats were hemosiderosis of spleen and liver and (in one cat) of the duodenal epithelium. These findings were attributed to the anemia, which varied widely between animals of one group.

The authors could discover 22 cases of "idiopathic oxalosis in man confirmed by histologic studies." They review the available information on the subject and include a good bibliography.

Gershoff and his colleagues point out the



similarity between "idiopathic" human oxalosis and the disease in their cats. The oxaluria and renal lesions are quite similar, as can be seen from their photomicrographs. The authors believe that the lack of calculi in the cats may be due to the short experimental period. Likewise, they believe that the absence of oxalate deposits in various other tissues, usually found in human cases, may be similarly explained, and this seems reasonable.

Gershoff *et al.* state that, in rats deficient in vitamin B<sub>6</sub>, they have consistently found "endogenous oxalate production accompanied by urinary tract calculi . . . with obstructive sequelae similar to those seen in human beings. . . ." They point out that experimental oxalate-induced renal lesions in other species are rather different from those

in the pyridoxine-deficient cats, consisting chiefly of tubular necrosis with associated crystal deposits.

Lastly, the authors suggest that pyridoxine deficiency (or metabolism) may be involved in human hyperoxaluria and oxalosis, in view of evidence that pyridoxine deficiency can occur in man. Not only has this deficiency been found in infants on a commercial formula diet (*Nutrition Reviews* **18**, 136 (1960)), but evidence exists that there are patients with abnormally large requirements of the vitamin.

Gershoff and his co-workers have evidently demonstrated that vitamin B<sub>6</sub> deficiency in the cat (and rat) leads to hyperoxaluria and oxalosis. This may very well be relevant to the human disease; one hopes that the thesis will soon be tested in patients.

### CHOLESTENONE FEEDING AND ARTERIOSCLEROSIS

*The feeding of delta-4-cholestenone to chickens resulted in pathological changes in aortas, liver and adrenal glands. Changes in sterols of plasma and liver were observed. Crystalline material from the aortic plaques was not cholesterol.*

Feeding diets containing large amounts of cholesterol results in arterial damage in experimental animals (*Nutrition Reviews* **13**, 20, 189 (1955)). The observation of G. M. Tomkins, H. Sheppard and I. L. Chaikoff (*J. Biol. Chem.* **203**, 781 (1953)) that feeding delta-4-cholestenone to rats decreased the amount of incorporation of C<sup>14</sup> of acetate-1-C<sup>14</sup> into cholesterol prompted studies of the effect of feeding delta-4-cholestenone on arteries and lipids of birds (C. W. Nichols, Jr., S. Lindsay, D. D. Chapman and I. L. Chaikoff, *Circulation Research* **8**, 16 (1960)).

White leghorn cockerels, seven weeks of age were divided into two groups and fed chow with 2.5 per cent cottonseed oil added. One group was given delta-4-cholestenone as 0.5 per cent of the diet. Food consumption was less in the cholestenone-fed birds after the first month.

Birds were killed each month and sections of aorta, liver, kidney and adrenal were

stained and lesions were studied grossly and microscopically. Total cholesterol and total digitonin precipitable sterols (TDPS) in the blood and tissues were determined and the difference, sterols not reacting with Lieberman-Burchard reagent (non-LBRS), was calculated.

Total plasma cholesterol fell in the cholestenone-fed birds as early as one month, but the plasma concentration of TDPS was elevated. The concentration of TDPS became elevated in the livers of cholestenone-fed birds. Cholesterol concentration of liver did not increase, but total liver cholesterol increased especially when the cholestenone had been fed three to five months.

A liver sample from one of the birds on the cholestenone diet was used in an attempt to characterize the sterols present. After hydrolysis, extraction of neutral lipids and precipitation using digitonin, the sterols were again released from digitonin and a sample

of dihydrocholesterol-4- $C^{14}$  was added. The samples were counted and gave a specific activity of 1200 counts per minute per mg. Then, by means of chromic acid oxidation, cholesterol and other unsaturated sterols were destroyed and dihydrocholesterol was converted to cholestenone and purified. The specific activity became 1730 counts per minute per mg., indicating that 69 per cent of the original sterol mixture had been dihydrocholesterol. This value is similar to the non-LBRS per cent of TDPS. The investigators concluded that the non-LBRS fraction was dihydrocholesterol.

Since cholestenone reduces the level of cholesterol in plasma it is potentially a therapeutic agent unless it tends to accumulate in tissues. In order to determine this point, liver and plasma samples from control and cholestenone-fed birds were extracted and fractionated. Neither the plasma nor liver contained more than 1 mg. per cent of cholestenone.

Gross examination of tissues of control birds showed lesions in the thoracic aorta in four of the 12 birds. All but one of the abdominal segments had plaques. Seven of the birds fed cholestenone had thoracic aorta lesions and all but one had abdominal aorta lesions. Livers of the cholestenone-fed birds were yellow-gray and enlarged. The gall bladders of control birds averaged 1.6 g. in weight, whereas in cholestenone-fed animals gall bladders averaged 8.6 g.

Histologic examination of the control thoracic aortas showed no intimal lesions.

Abdominal aortas all had lesions. Liver structure was normal. Adrenal cortical cells contained a few crystals resembling cholesterol. Kidneys were normal.

Cholestenone-fed birds had no intimal lesions in the thoracic aortas. Some lipid-staining material was present in the media in two of the birds. The abdominal aorta lesions, which were found in all but two of the birds, differed from those in the control group in that foam cells and lipid droplets were present. Elongated crystals were visualized but were probably not cholesterol. Liver cells appeared to be distorted by macrophages. Sudan IV-staining material was abundant but cholesterol was absent, as determined by the Schultz reaction.

Cortical cells of the adrenals of cholestenone-fed birds contained more lipid material. Refractile substances and Schultz positive materials were less.

The renal glomeruli were normal but tubular epithelial cells were swollen in cholestenone-fed birds. Sudan IV-staining material was present in the glomeruli and tubular epithelial cells and in the endothelium of some renal arteries.

The results as presented indicated that feeding delta-4-cholestenone does not prevent the development of atherosclerosis in the rabbit. Furthermore, there is evidence from these experiments that renal hepatic and adrenal changes result which could produce deleterious effects upon the function of these organs.

## NOTES

Symposium on Hereditary  
Metabolic Disorders

A. E. Garrod developed a concept of inborn errors of metabolism more than 50 years ago. Since then the four originally described inborn errors have increased to more than 50 hereditary metabolic diseases. A symposium on Hereditary Metabolic Diseases with G. E. Guest as special editor has been published recently (*Metabolism* 9, March and April issues (1960)). The following is a list of subjects reviewed:

*Hereditary Metabolic Diseases—General Considerations.* B. H. Landing.

*Hereditary Variations in the Synthesis of Serum Proteins in Health and Disease.* A. G. Bearn.

*Chemistry of Disease of the Central Nervous System.* J. N. Cumings.

*Inherited Defects of Thyroid Hormone Synthesis and Metabolism.* R. M. Blizward.

*Roentgen Manifestations of Hereditary Metabolic Diseases in Childhood.* F. N. Silverman and G. Currarino.

*Inherited Variations in Aromatic Metabolism.* H. E. Sutton and R. E. Tashian.

*Recent Advances in Biochemical Detection of Heterozygous Carriers in Hereditary Diseases.* D. Y. Hsia.

*Galactosemia.* H. N. Kirkman.

*Pathophysiology of Congenital Adrenal Hyperplasia.* W. R. Eberlein and A. M. Bongiovanni.

*Phosphatase Studies in Gaucher's Disease.* A. C. Crocker and B. H. Landing.

*Individual Metabolic Patterns: (I) Amino Acid Excretion Studies in 700 Children; (II) Excretion of Beta-Aminoisobutyric Acid.* H. K. Berry.

*Indicanuria in Phenylketonuria.* S. P. Bessman and K. Tada.

*Idiopathic Infantile Hypoglycemia and Leucine Sensitivity.* W. Cochrane.

## West German Nutrition Research

Nutrition research in West Germany is carried out largely by scientific institutes financed entirely or partly by the Federal Ministry of Food, Agriculture and Forestry (*German Science Bulletin* 40/41, 45 (1959)). Some of these institutes conduct research on topics of general interest such as quality of vegetable foodstuffs, food chemistry, food preservation, food technology and packaging, nutritional physiology and domestic science. Others limit their interests to more narrow fields such as research in milk, fish or fats.

These institutes have striven to develop a comprehensive research program. In the area of vegetable foods, this program includes the development of suitable plant strains, the study of the influence of fertilizers and insecticides on plants, measures to improve the biological value of vegetable foods and basic research on the structure and biochemical properties of vegetable foodstuffs. In the area of animal foods, the program includes basic research on quality in animal foods in conjunction with animal breeding research, the study of such production factors as the transport and treatment of animals, slaughtering and fish catching methods, food quality, the structure and biochemical properties of animal foods, the formation of proteins and the protein-fat ratio in animal foods. Modern methods of handling and processing, storage and solving problems of seasonal supply and surpluses are being studied with respect to both animal and vegetable foods.

Included in the general research assignments of the several institutes are investigations of the effects of preservatives, residues of plant protectives and other additives on foodstuffs, of food processing methods such as heating and irradiation and of animal and plant diseases.

In West Germany, there are 13 scientific institutes devoted to research on nutrition.

Together they form the Arbeitsgemeinschaft Ernahrungswissenschaftlicher Forschungsinstitute—AEI (Working Society of Research Institutes for Nutrition).

In an effort to avoid duplication and overlapping of projects and to attack certain problems through the work of research teams, the AEI annually drafts and coordinates the research plans of the various institutes. About 320 projects were so handled in the past fiscal year. Priority is given to projects of special urgency. For example, in the past year research connected with the new federal food laws received priority. Work was designed to study methods of keeping chemical preservatives and additives to a minimum and of preserving food by harmless methods. Priority was also given to the development of ready-to-cook and ready-to-eat products.

Other examples of the research projects assigned by the AEI during the past year are: (1) Influence of food coloring agents on the enzymes of the digestive tract and body cells. (2) Influence of freezing and storage conditions on the quality of frozen foods. (3) Studies on the chemical and physiological changes in industrial drying of proteins. (4) Research on the use of ascorbic acid as a partial substitute for nitrite in the pickling of meat. (5) Investigations of food preservation by physical processes (ultraviolet radiation, gamma radiation, x-rays, high frequency heating). (6) Methods of testing packaging materials and packages. (7) Mutual influences of packaging, contents of package and outside factors.

This program of coordinated research in West Germany resembles in many respects

the programs of individual government agencies in the United States. The direction given by the AEI is apparently much firmer than that imposed in most coordinated research programs in nutrition on the American scene. The success of such direction in the West German nutrition program is something to be watched with interest over the next few years.

### Nutrition for Man in Space

With the development of vehicles in which to send man into outer space the development of nutritional programs to sustain him becomes an important need. Recently, reports of lectures in aerospace medicine given in January 1960 have been made available by the School of Aviation Medicine, USAF Aerospace Medical Center, Brooks Air Force Base, Texas. A lecture by B. E. Welch entitled "Space Logistics: Food, Water and Waste" should be of interest to many nutritionists. In addition to the weight, volume and nutritional quality of foodstuffs, Welch discusses the recycling of water, a problem seldom considered by those interested in feeding programs.

### Recent Books

*Land for the Future.* By Marion Clawson, R. Burnell Held and Charles H. Stoddard. Published by The Johns Hopkins Press, Baltimore 18, Maryland. Pp. 500. Price \$8.50.

*Fundamentals of Nutrition.* By E. W. Crampton and Lewis E. Lloyd. Published by W. H. Freeman & Co., 660 Market Street, San Francisco 4, California. Pp. 494. Price \$7.50.

# NUTRITION REVIEWS

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## THE PHYSIOLOGICAL BASIS OF THIRST

### *The Internal Environment*

Evaluation of the internal environment conditions which are critical in triggering thirst continues to present a challenging experimental problem. The "dry mouth" theory of fluid intake has all but been abandoned, while notions of a generalized dehydration state are somewhat lacking in specificity. At the turn of the century, André Mayer, on the basis of cryoscopic observations, proposed that the increase in blood osmotic pressure following water deprivation was the stimulus to thirst.

With experiments such as A. Gilman's (*Am. J. Physiol.* **120**, 323 (1937)), it became clear that an increment in the effective osmotic pressure of extracellular fluid produces thirst. Gilman found that an intravenously administered, hypertonic sodium chloride solution elicited twice the water intake that a urea solution of the same tonicity did. The sodium chloride remains in the extracellular fluid space, while urea diffuses freely across cell membranes. J. H. Holmes and M. I. Gregersen (*Ibid.* **162**, 326 (1950)) showed that it was indeed the increase in effective osmotic pressure that was critical, for the thirst effect was not specific to serum sodium increments but could be produced by other substances, such as sorbitol, which did not cross cell membranes.

At this time, E. B. Verney (*Proc. Roy. Soc. (London)* **B135**, 25 (1947)), investigating the factors releasing antidiuretic hormone, also concluded that a small increment in the effective osmotic pressure of the blood supply to the supraoptic and paraventricular nuclei of the hypothalamus was an effective stimulus. Verney postulated the existence of osmoreceptors within these nuclei which detect changes in the effective osmotic pressure of the blood and regulate

the release of antidiuretic hormone accordingly. Then B. Andersson (*Acta Physiol. Scandinav.* **23**, 188 (1953)) demonstrated that this mechanism was related to water intake as well as renal conservation. Hydrated goats given minute intrahypothalamic injections of hypertonic sodium chloride drank copiously, while injections of isotonic sodium chloride produced no thirst.

Uniformity of osmotic pressure between intra- and extracellular space is regulated, not by the passage of ions, but by the exchange of water between these compartments. Any increase in extracellular fluid osmotic pressure, therefore, dehydrates the cellular space. The experiments discussed thus far cannot distinguish which of these internal events might be crucial as a stimulus to thirst: (1) increment in serum effective osmotic pressure, or (2) the consequent increase in intracellular osmotic pressure or shrinkage of cellular space. While it is not possible to dehydrate these compartments independently, it is possible to overhydrate the cells by depleting the extracellular fluid of its normal amount of electrolyte. By the technique of intraperitoneal dialysis, large quantities of sodium can be removed rapidly from the extracellular fluid. The resulting osmotic gradient produces a movement of water from the extracellular fluid into the cells.

If increased extracellular fluid effective osmotic pressure were the sole condition triggering thirst, we should expect decreases in water intake. If intracellular dehydration were the critical stimulus, we should also expect intake decreases. However, experiments on rabbits, rats, and dogs have shown increases in thirst following dialysis. This is probably the result of the decrease in extracellular fluid volume produced by dialysis. The question of the mechanism of

extracellular fluid volume reception and adjustment, which has become a central problem in the explanation of renal regulation, also has become a puzzle on the intake side of the ledger.

Much clinical evidence attests to the fact that salt depletion is associated with increased thirst. The thirst occasioned by salt losses from sweating in hot climates and which cannot be assuaged by drinking plain water, but is relieved by salt tablets, is well known. However, not all clinical views relating salt depletion and thirst are in agreement. H. L. Marriott (*Water and Salt Depletion*, p. 41. Charles C Thomas, Springfield, Ill. (1950)) states: "...there is no thirst; indeed, water is often repellent. I have seen men suffering from severe secondary dehydration (*i.e.*, salt loss) refuse water or spit it out." Such clearly contradictory reports doubtless indicate that the internal environment makes more distinctions about states of water-electrolyte depletion than do investigators and theorists.

While both increased serum effective osmotic pressure and decreased extracellular fluid volume are thirst stimuli, not all conditions giving rise to thirst can be simply reduced to these two alternatives. The excessive water intake of patients in a state of water intoxication, where serum osmolality is low and extracellular fluid volume is increased, remains unexplained (V. Wynn and C. G. Rob, *Lancet* I, 587 (1954)). Likewise, the precise stimulus for the massive fluid intake characteristic of diabetes insipidus remains obscure. Surgical destruction of the median eminence of the hypothalamus and the supraopticohypophyseal tract interferes with the elaboration of antidiuretic hormone. Logically enough, the resulting diuresis is followed by increased intake, but it is nonetheless disconcerting to learn that in diabetes insipidus there is no increment in serum sodium concentration, and extracellular fluid volume is expanded (S. M. Friedman *et al.*, *Am. J. Physiol.* **192**, 401 (1958)). What, then, is the internal

environment condition sustaining thirst in diabetes insipidus?

### *Hormonal Factors*

F. C. Bartter and his associates have shown that the secretion of aldosterone can be controlled by extracellular fluid volume independent of concentration; increases in extracellular fluid volume decrease secretion, while decreases in volume increase secretion. It is possible that the extracellular fluid volume-regulating aspect of thirst is a function of aldosterone level. Although desoxycorticosterone acetate administration does give rise to polydipsia, this has been interpreted as secondary to salt retention, rather than a direct hormonal sensitization of neural "drinking centers." Nor does pitressin seem to have any direct action on thirst mechanisms in either normal or diabetes insipidus animals.

The most promising evidence for the existence of a "thirst hormone" is the report by G. J. Gilbert (*Am. J. Physiol.* **191**, 243 (1957)). Electrolytic destruction of the subcommissural area in rats produced a permanent adipsia resulting in death from dehydration. Injection of subcommissural area extract into normal rats resulted in a sharp but temporary depression in daily water intake.

### *Metabolic and Regulatory Polydipsia*

Stimulation of limited areas in the hypothalamus by means of permanently implanted electrodes elicits polydipsia, while destruction of such areas produces an adipsic animal. Various hypothalamic lesions also have resulted in chronic polydipsia. There exists no crucial test distinguishing a metabolic polydipsia, which is simply secondary to a pre-existing polyuric state, from a regulatory polydipsia, wherein the large urine volume is a consequence of high fluid intake. (Exceptions to this are the cases of genetic renal defect which are refractory to pitressin treatment.)



The occurrence of regulatory or primary polydipsia is inferred from diverse clinical reports. It is assumed to originate from a central nervous defect which perhaps mimics the effect of electrical stimulation of a hypothalamic "thirst center." The low glomerular filtration rate, which results from the enforced dehydration of a diabetes insipidus patient, allows a quite efficient renal conservation of water, so that urine values are by no means an infallible guide to the evaluation of metabolic polydipsic states. Again, animals with different polydipsogenic hypothalamic lesions all yield a diabetes-insipidus picture when subjected to a hypertonic saline challenge.

However, in evaluating neurohypophyseal disfunction, J. F. Dingman, K. Benirschke and G. W. Thorn (*Am. J. Med.* **23**, 226 (1957)) have obtained differential patterns of responsiveness to the antidiuretic stimuli, nicotine and hypertonic saline. The classification of such patterns holds the promise of an objective basis for distinguishing regulatory from metabolic polydipsia.

*Central Nervous System: Sampling the Internal Environment*

It has been shown that the blood-brain barrier is relatively permeable in certain delimited areas. Vital dyes such as trypan blue do not penetrate the barrier except at the following sites: supraoptic crest, intercolumnar tubercle, neurophyophysis and stalk, area postrema and the subcommisural area. It is undoubtedly significant that precisely these areas are intimately related

to the control of body water. We have already mentioned Verney's supraoptic osmoreceptors; here, too, C. von Euler has recorded "osmo-potentials" following intracarotid injection of hypertonic saline. The neurohypophysis and stalk, in conjunction with the hypothalamus, elaborates, stores, and releases antidiuretic hormone.

Gilbert's experiments on the subcommisural area, previously described, have led him to postulate the elaboration of a thirst hormone in this area, and to consider the possibility that this region is the much sought "volume receptor." In recent work, he has shown histological changes in this area resulting from water deprivation. G. Farrell and his co-workers demonstrated that lesions and extracts of this area reduce and stimulate aldosterone secretion respectively. Finally, C. D. Clemente, J. Sutin and J. T. Silverstone (*Am. J. Physiol.* **188**, 193 (1957)) recorded electrical changes just lateral to the area postrema in the medulla upon intravenous injection of hypertonic saline. These were interpreted as "osmo-potentials."

We suggest that these central areas, which are permeated by trypan blue, form a system which samples the effective osmotic pressure and perhaps the volume of the blood, and integrates this information into the adjustive actions of renal regulation and intake behavior.

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## FOOD CONSUMPTION IN A HOT DESERT ENVIRONMENT

*The caloric needs of military personnel carrying out light activities in a hot desert environment were found to be similar to caloric requirements under temperate conditions.*

During the past five years the United States Army Medical Research and Nutrition Laboratory has been conducting nutritional surveys which included studies at five army camps and seven hospitals

throughout the United States. Also studies have been performed under extreme subarctic conditions with troops performing heavy activities. Recently, C. F. Consolazio and associates (*Metabolism* **9**, 435 (1960))

attempted to establish the minimal energy requirements and also the nutritional status of troops performing light activities while living under extremely hot desert conditions. The studies were carried out at the Yuma Test Station, Yuma, Arizona in 1957.

Two groups of army personnel were used, one comprising the military police and the other the remaining headquarter enlisted personnel, who carried out light clerical activities. Food consumption at the army mess was measured for the entire group of individuals, but not on an individual basis. In addition, the quantity of food consumed from outside sources was computed. Weekly weighings determined any change in body weight. The food was prepared by regular army cooks and bakers who used the Master Menu as a guide for preparation, and the investigators did not change or interfere with either the type of food offered or the quantity.

The protein, fat and carbohydrate contents of the foods were determined by chemical means and the caloric content calculated from the diet composition. Samples were collected from five representative servings of each food item offered at each meal and these were subjected to chemical analysis. At the end of each meal all the edible foods left on the plates were combined, homogenized and analyzed. Thus, by knowing the total amount of food served, the amount returned and the number of persons eating at any given meal, it was possible to calculate how much fat, protein and carbohydrate an average individual ate.

In order to determine the amount of food eaten outside the mess, a questionnaire containing a list of all popular food items was given to each individual, who recorded the amount consumed each day. (Previous studies by this group have indicated that approximately 17 per cent of the total food consumption of military personnel is obtained outside the mess.) The protein, fat, carbohydrate and caloric content of these supplementary foods was obtained by using standard nutritional tables.

Three periods of food consumption were measured. For the first period lasting seven days, the food consumption of the headquarters and military police groups was measured separately. After one free day, the food intake during a six-day period again was measured for the separate groups. Finally, a six-day study period was set up in which the amount of food consumed for the combined groups was computed.

During each of the three study periods, the mean daily temperature averaged 93, 95 and 97°F with the maximum temperatures being 111, 109 and 109 for the same periods. The relative humidity for the three periods averaged 21 to 28 per cent. There were 238 persons studied in the headquarters personnel group during the first period and 214 during the second, while the military police group averaged 53 members during both periods. It should be noted that the average total food consumption from all sources for the headquarters personnel averaged 3999 and 4130 calories for the two study periods. There was no change in the average body weight of this group during the two periods.

The military police groups, on the other hand, had caloric intakes of 4237 and 4595 calories per man per day during the first and second periods, respectively. In spite of their larger caloric intake, however, this group lost the equivalent of 17 g. per day during the 14-day study period.

R. E. Johnson and R. M. Kark (*Science* 105, 378 (1947)) reported that the caloric requirement of soldiers was affected by environmental temperature, and they concluded that the energy requirement of soldiers varies inversely with the mean environmental temperature. Thus the Dietary Allowances of the Food and Agricultural Organization and the National Research Council now provide for climatic differences, recommending a five per cent increase or decrease in the energy requirement for every 10°C decrease or increase in a mean environmental temperature of 20°C.

By this standard, the personnel at Yuma, Arizona, should have a 10 per cent decrease

in energy requirement. Assuming, that these personnel have an average body weight of 73 kg. and that the mean outdoor temperature is 95°F, the suggested caloric intake should be 3069 calories per man per day, a value considerably lower than the intake actually determined. However, the present investigators believe that the elevation in the expected caloric intake results from the extra energy needed to produce sweat, an essential factor for maintaining the body temperature in this hot environment.

The daily energy expenditure was not computed for each of the groups. However, the military police group spent at least eight to nine hours outdoors in the heat when on duty and thus lost considerable amounts of sweat, which may explain in part their greater food consumption and loss of body weight. Also when off duty the personnel spent a fair amount of their time swimming or playing softball.

Normally in hot climates there is a tendency toward a reduced caloric intake, but in the present study the mess hall was air conditioned and considerably cooler than

the outside environment. In addition to the cool atmosphere, large quantities of iced cantaloupe and watermelon, iced fruit juice and milk were available at each meal. Thus eating in the mess hall was always pleasurable. It is of interest that the high food consumption of the headquarters and military police group were in the same range as that of military personnel performing hard physical work at both a temperate environment (4265 calories per day) and in a sub-arctic environment (4163 calories per day). Thus this study indicates that there is no decrease in the required food intake of men caused by solar radiation and high environmental temperature.

It should be noted that about 40 per cent of the total calories consumed for both groups came from fat. This is in contrast to some studies which indicate that in tropical areas there is a low fat consumption. The present investigators believe that the increased fat consumption in their study was not due to environmental conditions but rather to food habits which did not change with the environmental temperature.

## HORMONE EXCRETION, BEHAVIOR PATTERN AND CORONARY DISEASE

*The urinary excretion of certain hormones was studied in an attempt to determine why a behavior pattern characterized by competitive activity and a sense of time urgency alters the incidence and intensity of blood and cardiovascular abnormalities.*

Several studies have been published recently indicating that periods of occupational stress are associated with elevated blood cholesterol levels and, in some cases, increased rate of blood clotting (M. Friedman, R. H. Rosenman and V. Carroll, *Circulation* **17**, 852 (1958); P. T. Werlake *et al.*, *Proc. Soc. Exp. Biol. Med.* **97**, 163 (1958); C. B. Thomas and E. A. Murphy, *J. Chron. Dis.* **8**, 661 (1958); S. M. Grundy and A. C. Griffin, *Circulation* **19**, 496 (1959)).

Rosenman and Friedman (*J. Am. Med. Assn.* **169**, 1286 (1959)) have recently compared a group of men exhibiting chronic "excessive and competitive drive and an enhanced sense of time urgency" (pattern

A) with a group exhibiting the opposite type of behavior (pattern B). It was found that the men with behavior pattern A, besides having a faster clotting time and higher serum cholesterol, also had about seven times higher incidence of clinical coronary artery disease and more than three times as much arcus senilis (a ring of degeneration about the corneal periphery). These investigators also found that females with behavior pattern A, when compared with females exhibiting pattern B behavior, had similar differences in blood and cardiovascular characteristics (*Circulation* **20**, 759 (1959)).

Since these different types of individuals had similar diets and physical activity, Friedman, S. St. George, S. O. Byers and Rosenman (*J. Clin. Invest.* **39**, 758 (1960)) studied the urinary excretion of possibly relevant hormones in an effort to determine how lipid changes might be induced by behavior pattern A.

Men between the ages of 35 and 50 were selected for this study on the basis of three main criteria. First, their occupations were associated with extreme competitive activity and preoccupation with deadlines; second, the individuals stated that they felt a sense of competitive drive and time urgency; and third, all men exhibited by their actions a severe sense of time urgency. Men with characteristics opposite to these were selected to represent pattern B behavior.

All subjects had normal blood pressure and were free of subjective symptoms of clinical coronary artery disease.

Urine was collected during working hours in order that the products of excretion would correspond to the time when the behavior pattern was most likely to be present. One urine sample was collected following bed rest. Each subject was asked to estimate his intensity of time urgency during the four days of urine collection.

The hormone assay included 17-ketosteroids, 17-hydroxycorticosteroids, 5-hydroxyindole, epinephrine and norepinephrine.

Records of dietary, tobacco, alcohol, work and exercise habits were made. Data were collected on serum cholesterol, blood clotting, and the incidence of arcus senilis and clinical coronary artery disease.

The number of working hours and the amount of physical activity were greater for the men in group A, but the exercise tended to be more concentrated on weekends. Alcohol and cigarette use was much greater in this group. Total calories, carbohydrate, protein and fat intakes were essentially similar in both groups.

Average serum cholesterol of group A (276 mg. per cent) was significantly higher than the average for group B (209 mg. per cent). Similarly, clotting time was significantly faster in group A (6.4 minutes) than in group B (7.2 minutes).

Arcus senilis was present in eight of the 12 men from group A and in none from group B. Four of the men in group A and none from group B had clinical but asymptomatic coronary artery disease.

The excretion of 17-hydroxycorticoids increased in both groups during working periods. The urinary excretion of 17 ketosteroids while working was about the same in both groups. The 5-hydroxyindole excretion did not differ between night or working hours or between groups.

Excretion of epinephrine increased markedly during working hours in both groups. Men of group A showed an increase of 86 per cent as compared with a 36 per cent increase for group B, but the difference was not statistically significant. On the other hand, men in group A showed an increase of 173 per cent in excretion of norepinephrine during working hours as compared with a 64 per cent increase for men of group B, and this difference was statistically significant.

In the opinion of the authors, two factors were responsible for the production of the behavior pattern A, namely, the intensity of the environment and the individual reaction to it. Modification of either factor could conceivably alter the biochemical response. They believe that any attempts to confirm these results should be characterized by rigid adherence to the proper conditions for the study of catecholamine excretion. In other words, if either the individual reaction or the special environmental conditions are lacking, the elevated norepinephrine excretion would probably not occur.

Whether a part-time discharge of excess catecholamines over a period of years could affect the pathogenesis of arterial disease is not known. Further studies correlating the

incidence of coronary artery disease with the presence of behavior pattern A would contribute to a better understanding of this problem. If atherosclerosis in experi-

mental animals could be made more severe by administering norepinephrine, it would significantly implicate this compound in a causative rather than merely associative role.

### METABOLIC CHANGES ACCOMPANYING LOW VITAMIN B<sub>6</sub> INTAKES

*Changes occurring in college students on a low vitamin B<sub>6</sub> intake suggest that xanthurenic acid excretion after a tryptophan test dose, lymphocyte count and blood pyridoxine may indicate nutritional status for vitamin B<sub>6</sub>.*

The syndrome of a vitamin B<sub>6</sub> deficiency in man has not been clearly described. However, recent investigations, such as that by R. W. Vilter *et al.* (*J. Lab. Clin. Med.* **42**, 335 (1953)) of vitamin B<sub>6</sub> deficiency produced by feeding desoxypyridoxine and that by A. D. Hunt (*Am. J. Clin. Nutrition* **5**, 561 (1957)), have suggested that a deficiency of vitamin B<sub>6</sub> in man may be more prevalent than previously supposed. This assumption is partly based on the observation that a deficiency of pyridoxine in the human being may mimic changes occurring in deficiencies of other B vitamins. Unfortunately, the number of tests for vitamin B<sub>6</sub> deficiency in the human subject are limited and thus the changes in xanthurenic acid excretion following a test dose of tryptophan and the changes in blood vitamin B<sub>6</sub> concentration and in the lymphocyte count in the blood may be more suggestive than definitive in evaluating vitamin B<sub>6</sub> deficiency.

K. E. Cheslock and M. T. McCully (*J. Nutrition* **70**, 507 (1960)) have endeavored to follow the changes occurring in human subjects maintained for up to 52 days on a diet low in vitamin B<sub>6</sub>. Their experiments were carried out on college students, seven women and one man, ages 18 to 20, who were fed a diet of natural foods found upon analysis to be low in vitamin B<sub>6</sub>. The diets provided the women a daily average of 0.414 mg. of vitamin B<sub>6</sub> and 4.22 g. of nitrogen while the man received 0.50 mg. of vitamin B<sub>6</sub> and 5.75 g. of nitrogen per day.

Vitamins B<sub>1</sub>, B<sub>2</sub>, niacin, and 7 mg. of iron were added in order to insure that these

dietary constituents were not inadequate. The diets supplied an adequate amount of energy and the subjects of this study showed very little weight change during the seven weeks of the experiment.

The only possible gross evidence of a vitamin B<sub>6</sub> deficiency was the complaint of excess fatigue on the part of some of the students. However, Cheslock and McCully observed that this complaint is a common one on the part of college students and may well be due to other factors.

Four weeks after the vitamin B<sub>6</sub>-deficient diet was started, pyridoxine in the blood had dropped to zero and remained at this level until the end of the diet period. Administration of 100 mg. pyridoxine hydrochloride upon termination of the experimental diet resulted in a rapid increase in vitamin B<sub>6</sub> content of the blood to a level several times that found eight weeks later. There was a considerable variation in the blood content of vitamin B<sub>6</sub>, ranging from 1.28 µg. per 100 ml. to 14.94 µg. per 100 ml. in a subject who had been suffering from a thymoma.

In five of the eight subjects, a 5 g. test dose of L-tryptophan resulted in an excretion of more than 30 mg. of xanthurenic acid per day, which is above the level at which a deficiency of vitamin B<sub>6</sub> may be thought to exist. Two other subjects exhibited increased excretion of xanthurenic acid, but at a lower level than 30 mg. The male subject showed very little change in xanthurenic acid excretion. Since he had received somewhat more vitamin B<sub>6</sub> than the women, it

was suggested by Cheslock and McCully that perhaps this amount more nearly met his requirements.

The blood studies were characterized by a decrease in lymphocyte count in five of the eight subjects. This decrease showed some significance and may have been the best evidence that a vitamin B<sub>6</sub> deficiency was developing. The subject with a thymoma did not show appreciable change in lymphocyte count.

The rapid drop of vitamin B<sub>6</sub> in the blood without other severe changes to indicate vitamin B<sub>6</sub> deficiency suggests that the human being may have a priority system respecting the use of pyridoxine within the body. The fact that, following tryptophan administration, three subjects did not experience a rise in xanthurenic acid excretion to a level suggestive of vitamin B<sub>6</sub> deficiency may mean that they were receiving sufficient

vitamin B<sub>6</sub> to protect them from this metabolic change. Cheslock's earlier work with rats (*J. Nutrition* **65**, 53 (1958)) indicated that perhaps twice the normal requirement of pyridoxine must be fed before vitamin B<sub>6</sub> can be detected in the blood.

In evaluating the results presented by Cheslock and McCully it appears that young college women probably need more than 0.5 mg. of pyridoxine per day. However, it is also apparent that, at least for comparatively short periods of time, a diet containing less than this amount will not produce obvious signs of vitamin B<sub>6</sub> deficiency. Certainly this work emphasizes the need for more definitive methods of analysis to permit an accurate assessment of the metabolic changes occurring with low pyridoxine intakes. This is especially true if an effort is to be made to identify a sub-clinical deficiency of this vitamin in human subjects.

### UTILIZATION OF FRUCTOSE BY WORKING MUSCLE

*In a patient with an hereditary metabolic defect who developed muscular weakness on exercising, it was found that fructose could be utilized by the working muscle.*

By studying the arterial-venous difference following an intravenous infusion of fructose, T. B. Van Itallie and associates (*Proc. Soc. Exp. Biol. Med.* **84**, 713 (1953)) and M. Miller *et al.* (*Yale J. Biol. Med.* **29**, 335 (1956-57)) obtained evidence to indicate that there was an uptake of fructose by the muscles of the forearm. This could be interpreted as utilization of fructose by these muscles.

Recently, C. M. Pearson and D. G. Rimer (*Proc. Soc. Exp. Biol. Med.* **100**, 671 (1959)) studied a patient who had a rare muscle disorder, previously described by B. McArdle (*Clin. Sci.* **10**, 13 (1951)). This individual was unable to continue moderate muscular activity for more than four minutes. Beyond this time there was rapid fatigue, followed by paralysis. It was noted that ischemically exercised forearm muscles

became rapidly fatigued and there was no measurable increase in pyruvic or lactic acid levels in the venous blood draining the exercised arm. Normal individuals subjected to the same exercise had a fourfold rise in pyruvic and lactic acids in the venous blood coming from the tested arm. In an addendum, these investigators reported that the muscular defect in this individual was an absence of phosphorylase a and b.

For this study, the subject was exercised in the fasting state on a treadmill set at a 10 per cent grade and moving at the rate of four miles per hour. He received intravenously at different times normal saline, sodium bicarbonate, 10 per cent glucose or 10 per cent fructose solutions. The sugars were given at the rate of 0.73 g. per kg. per hour for 30 minutes prior to as well as throughout the exercise. In 20 test periods,



during which time no intravenous alimentation was given, it was found that the patient could walk on the treadmill for no longer than four minutes; the average duration of work was three minutes 20 seconds. Administration of saline or sodium bicarbonate did not improve the ability to perform.

The exercise tolerance, however, could be increased 20 times (the limit of the stress) by giving glucose intravenously during the exercise, the improved capacity occurring when the plasma glucose levels reached 160 mg. per cent and above. Upon administration of fructose, there was a similar improvement in the ability to exercise without fatigue so that walking could be continued for 60 minutes. In this case there was no significant increase in the plasma glucose level, but the fructose level rose to a maximum of 35 mg. per cent. When the infusion was stopped and the exercise continued it was possible for the patient to walk another 23 minutes, after which rapid fatigue and paralysis of the muscles of the legs developed. At this time the plasma fructose levels had fallen to 8 mg. per cent.

This study indicates rather clearly that fructose can increase the work capacity of muscles. It also suggests that the mechanism of action does not involve the conversion of fructose to glucose, with the latter serving as the direct source of energy, since there was no significant change in the plasma glucose levels during fructose administration. It was shown that, for glucose to be effective, the plasma glucose level must be about 160 mg. per cent, a value which did

not occur following the administration of fructose.

It also appears from this study that fructose levels remain constant during exercise. Moreover, the entrance of fructose into the muscle occurs at a much lower level than for glucose, since at an extracellular fluid fructose level of about 10 mg. per cent muscular work could still be performed, whereas plasma glucose had to be increased to nearly twice the fasting level before work could be carried out by the muscle.

Another explanation for the ability of fructose to increase the work capacity of muscle is found in the abstract of Pearson, Rimer and W.F.N.M. Mommaerts (*Clin. Research* 7, 298 (1959)). Using the same individual as reported earlier, they noted that in addition to glucose and fructose, lactate when given intravenously would increase the ability to exercise without fatigue. The possibility thus exists that lactate is the substance actually being utilized by the muscle, and hence the reason that fructose is effective at a lower blood level than glucose is that fructose is a much better lactate former. In the present study, however, one cannot establish whether fructose is used directly by the muscle or whether it is first converted to lactate so that the latter is the substance utilized.

Thus by observation of a young male subject with a metabolic disorder in which there appeared to be a failure to form lactic acid from glycogen under anaerobic conditions, it was found that the muscles can utilize fructose for energy purposes.

## POSSIBILITY OF A NEW PITUITARY LIPEMIA-PRODUCING HORMONE

*Injection of pituitary extract produces hyperlipemia in fed rabbits. A fraction has been prepared, free of the recognized pituitary hormones. Adrenocorticotrophin enhances its activity.*

Not often is a new pituitary hormone demonstrated nowadays, but this may well have been accomplished in the studies of D. Rudman and others.

Rudman and F. Seidman became interested in "Lipemia in the rabbit following injection of pituitary extract" and their first paper, with this title, appeared in

1958 (*Proc. Soc. Exp. Biol. Med.* **99**, 146 (1958)). Previous work of others had suggested that injection of pituitary substances leads to mobilization of depot fat and a rise in plasma non-esterified fatty acids, with liver fat deposition, increased fatty acid oxidation and formation of ketones.

Specifically, anterior pituitary extract and adrenotropic, growth or thyrotropic preparations had been demonstrated to increase liver fat; injection of growth hormone had been shown to cause an increase in plasma non-esterified fatty acids.

With these and related observations as a background, Rudman and Seidman studied the effect of pituitary extracts on serum lipids in the rabbit. Whole pituitary glands (lyophilized or acetone-desiccated) were extracted in the cold with one-tenth molar disodium phosphate. The rabbits were males weighing 2.5 to 4 kg., fed standard rabbit chow. They received a single subcutaneous injection of the pituitary extract and were not fasting except for blood sugar determinations.

One injection of extract from 100 mg. of lyophilized whole hog pituitary caused gross lipemia in 16 of 20 rabbits. Serum cholesterol and total lipids increased within six hours and rose sharply to a maximum in 18, usually returning to normal within 48 hours. The peak values shown for one rabbit were about four times the pre-injection level of serum cholesterol and seven times that for total lipids.

The increase in total serum lipids and in cholesterol was a straight-line function of the logarithm of the dose of pituitary extract. Plasma non-esterified fatty acids increased two- to threefold 18 hours after injection. Fasting blood sugar levels and glucose tolerance were not affected.

Extracts derived from lyophilized glands were three to four times more potent than those from acetone-desiccated glands. Hog, sheep, beef and human glands were all active. The activity of a single anterior lobe was about seven times greater than that of a

single posterior lobe. Diffusion between lobes, as well as imperfect slaughterhouse separation, presumably accounts for the apparent activity of both lobes.

The six hormones of the anterior pituitary were individually tested in doses five or more times the quantities contained in the amount of pituitary extracted. No lipemia was detected at 18 hours, except in one of four rabbits receiving growth hormone.

Rudman and Seidman found their lipemia-producing material to have many of the physical characteristics of a protein. They concluded that they were dealing either with a distinct substance, or with a synergistic effect of two or more of the recognized pituitary hormones.

In the next publication of this group (except for two abstracts), Rudman, Seidman and M. B. Reid (*Proc. Soc. Exp. Biol. Med.* **103**, 315 (1960)) have extended their studies, seeking to determine whether the lipemia-producing activity is due to synergism between recognized hormones or to a different and unrecognized substance. They have succeeded by fractionation in separating a lipemia-producing component from the other recognized pituitary hormones.

Lipemia production was measured by the change in total serum lipids of the rabbit 18 hours after subcutaneous injection of the test material. Other hormone activities were assayed by standard methods.

Since 15 rabbits showed an increase in total serum lipids of  $50 \pm 142$  mg. per cent (after injection of 5 ml. of distilled water), the authors attributed significance only to increases of more than 380 mg. per cent ( $p > 0.01$ ).

The six recognized anterior pituitary lobe hormones were separately tested. The dosages of adrenocorticotrophin (ACTH), growth hormone (GH) and thyroid-stimulating hormone (TSH) corresponded to the amount of each hormone present in 0.5 g. or more of desiccated gland. Prolactin, interstitial-cell stimulating (luteinizing) hormone (ICSH) and follicle-stimulating hormone (FSH) were

tested in 25 mg. amounts. None raised total serum lipids significantly. Injection of an alkaline extract of 60 mg. of desiccated hog pituitary gland produced an increase in serum total lipids of  $1450 \pm 205$  mg. per cent. All 15 possible two-hormone combinations were tested, each hormone at half the dose used singly. Three pairs produced significant lipemia; these were ACTH plus TSH, prolactin or FSH. These experiments were repeated with purified TSH without effect, indicating that the synergism was between ACTH and some contaminating substance other than TSH. Purified prolactin and FSH were not similarly tested.

The authors next investigated whether synergism between ACTH and other components were responsible for the entire lipemia-producing activity of crude pituitary extract. Fractionation studies on acetone precipitates of a saline extract showed lipemia-producing activity only in the acetone 50 to 90 per cent fraction, and mainly in the 75 to 90 per cent fraction. Since the latter was previously known to be free from adrenocorticotrophin, growth hormone, prolactin and thyroid-stimulating hormone, it seemed clear that synergism of ACTH could account for only a part of the total lipemia-producing activity of the pituitary gland and that a substance different from ACTH, GH, TSH and prolactin was at work.

Rudman's group then devised a technique for concentrating the lipemia-producing substance. A saline extract of lyophilized intact hog pituitary glands was subjected to acetone fractionation in the cold. "Fraction G" yielded 15 mg. of material per gram of desiccated pituitary gland. Fraction G was then run through a column of a cation exchange resin and fractions were collected serially. Physiologic activity was found to be sharply concentrated in a few tubes containing about 30 per cent of the material put on the chromatographic column. The material in these tubes was pooled and precipitated by ammonium sulfate, dialyzed and lyophilized. 5 mg. of this material ("Fraction H") was

obtained per gram of desiccated pituitary gland.

Three milligrams of Fraction H caused a 200 per cent increase in rabbit serum lipid level. Eighty-five per cent of the increase consisted of triglycerides, 5 per cent of cholesterol and 10 per cent of phospholipids. The action of Fraction H was intensified by injection with ACTH. Assays of Fraction H for the six recognized anterior and two posterior pituitary hormones showed none present in more than 0.8 per cent concentration. A mixture of the eight hormones representing their maximal content in up to 25 mg. of Fraction H was without significant effect upon rabbit serum lipid levels.

The authors recovered only 10 per cent of the original activity in their final Fraction H, whether because of loss, inactivation, or removal of synergistic hormone(s), *i.e.*, ACTH. They discuss the possible role of synergism between the known hormones and the existence of a hypothetical unknown "contaminant," *i.e.*, their own lipemia-producing substance. Their work gives no clue as to the mechanism of the synergistic effect of ACTH on lipid metabolism, which, they point out, may not even be adrenal-mediated.

Rudman, Seidman and Reid conclude that "lipemia-producing activity of crude hog pituitary extract is caused by the combined actions of (a) lipemia-producing component in Fraction H; (b) ACTH; and (c) substances present in commercial preparations of TSH, prolactin and FSH."

The authors appear to have demonstrated a lipemia-producing substance distinct from each of the nine recognized pituitary hormones. Vexing questions come up, however. Why should any other pituitary hormone (*i.e.*, ACTH) have any synergistic effect? This seems contrary to general experience in endocrinology, although not impossible. It is unlikely that the lipemia-producing factor itself could be a contaminant of the ACTH, even though this seems to be the explanation of the difference between TSH and purified TSH in combination with ACTH.

There is evidence that ACTH has a direct effect on adipose tissue, perhaps even remaining after corticotrophic action is destroyed. M. C. Schotz, G. M. C. Masson and I. H. Page have studied the effect *in vitro* of ACTH on the release of non-esterified fatty acids from adipose tissue of adrenalectomized rats (*Proc. Soc. Exp. Biol. Med.* **101**, 159 (1959)). They found that ACTH increased the release of fatty acids, that prior adrenalectomy decreased the process, and concluded that "ACTH may have a physiological role in fatty acid mobilization not mediated by adrenal cortical tissue."

How does Rudman's lipemia-producing effect differ from the effect of growth hormone, which also reduces fat stores, increases liver fat and causes ketosis? Injection of growth hormone into the dog or man produces a striking rise in fasting plasma non-esterified fatty acids, as reported by M. S. Raben and C. H. Hollenberg (*J. Clin. Invest.* **38**, 484 (1959)). This effect was suppressed by glucose or food or glucose and insulin, and the authors concluded that growth hormone influences the rate of fat mobilization in the postabsorptive period.

The essential difference in the two systems is that Rudman's group is working with the fed rabbit, in which the late effect of growth hormone on plasma non-esterified fatty acids is suppressed by feeding. However, in spite of the experimental differences between the

two systems, there are some disconcerting similarities. Only much more work on the mechanisms involved will disclose whether these metabolic effects are in fact disparate. If they are, it seems strange. One might complicate matters further by asking whether Rudman's substance causes secondary changes in secretion of growth hormone or other substances concerned in fat mobilization.

The fat-mobilizing substance recently extracted from human urine by G. L. S. Pawan (*Biochem. J.* **74**, 29P (1960)) may be similar or identical to Rudman's lipemia-producing substance. Pawan's material occurred in the urine of fasting human subjects and the pituitary was "necessary for its production." On hydrolysis, it yielded seven amino acids. In the mouse it increased fat mobilization and produced loss of body weight and reduction in blood sugar. Pawan concluded that it was neither growth hormone nor adrenocorticotrophic hormone.

Rudman and co-workers seem to have demonstrated a new pituitary hormone which produces lipemia. The mechanisms of the lipid-mobilizing effects of this substance and of growth hormone and ACTH are insufficiently defined at present for differences and similarities to be pointed out with any assurance. Likewise the relation of Rudman's to Pawan's substance is not established.

### ALTERATION OF PROTEINURIA BY EXCESS FEEDING OF INDIVIDUAL AMINO ACIDS

*An excess of poorly metabolized amino acids force fed to the rat produces an alteration of proteinuria. D-Serine also causes kidney tubule changes.*

There has been much interest in the effects of prolonged feeding of an unbalanced diet and investigations have been carried out with both excesses and deficiencies of various dietary constituents. The importance of proteins and their constituent amino acids has been demonstrated by the changes

which occur upon feeding excesses of individual amino acids.

Proteinuria in the rat has been shown to increase as the quantity of nitrogen in the diet increases. As long ago as 1926, T. E. Addis, M. Mackay and L. L. Mackay (*J. Biol. Chem.* **71**, 139 (1926)) reported on

changes in the kidney resulting from prolonged feeding of high levels of protein and cystine. This relationship between nitrogen intake and proteinuria in rats has led to investigations of the effect of added levels of individual amino acids upon proteinuria. R. W. F. Hardy and C. A. Baumann (*J. Nutrition* **69**, 429 (1959)) demonstrated that increasing the level of dietary glycine resulted in a substantial decrease in proteinuria.

In a more recent study of the effects of a number of additional amino acids, Hardy, W. O. Tantengco and Baumann (*J. Nutrition* **70**, 438 (1960)) observed that, while in general an increase of nitrogen in the diet of the rat increases proteinuria, the individual amino acids may have very different effects. The D and L forms of the amino acids also may have different actions, possibly depending upon the ease with which these forms are metabolized by the animal.

These investigators used mature rats maintained on a diet containing 20 per cent casein as the protein source. The rats were given by stomach tube 140 mg. doses of some 24 different forms of amino acids and then fasted for 24 hours. Urine collections were partitioned by paper electrophoresis and starch-gel electrophoresis, and this information was supplemented by chemical analysis for various urinary constituents.

Of particular interest were the results obtained following the administration of DL-serine, D-serine and L-serine. The losses of amino acids into urine accounted for only a small portion of the total administered dose, but in the case of DL- or D-serine, losses of 16 and 10 mg. of alpha-amino nitrogen per rat per day, respectively, were observed. This compares with only 2.6 mg. following the administration of L-serine. The increase in urea upon administration of many of the amino acids reflected the metabolism of the nitrogen from the administered amino acids. However, it is

notable that this increase did not occur with DL or D-serine.

The increase in protein excretion in the urine upon administration by stomach tube of DL and D-serine has been observed by W. H. Fishman and C. Artom (*J. Biol. Chem.* **145**, 345 (1942)), who also observed the sparing action of vitamin B<sub>6</sub> against the adverse effect of the DL-serine (*Proc. Soc. Exp. Biol. Med.* **57**, 239 (1944)). Hardy, Tantengco and Baumann (*loc. cit.*) demonstrated that additions of pyridoxine at a level of 100 mg. per kg. of diet reduced the protein excretion produced by the administration of DL-serine, but they also observed that a 25 mg. per kg. addition of pyridoxine or the administration of an anti-metabolite, desoxypyridoxine, did not increase the protein excretion above that observed on the diet without added pyridoxine.

Experiments conducted by these investigators point up the inability of the rat to either metabolize or reabsorb from the tubule excretions large doses of D-serine. This was further confirmed by the observation that, following a single large dose of this amino acid, protein excretion in the urine remained high for at least three days.

The decreased urea excretion following administration of D-serine suggests an alteration in kidney function. Earlier work by R. P. Morehead, Fishman and Artom (*Am. J. Path.* **21**, 803 (1945)) and by M. Wachstein (*Arch. Path.* **43**, 503 (1947)) had indicated that the damage from this amino acid probably was localized in the distal region of the proximal tubule. Hardy, Tantengco and Baumann (*loc. cit.*), in extending their study to paper and starch-gel electrophoretic partitioning of the urine, observed that the protein excreted by rats fed excess DL-serine varied from the normal in that albumin became the major component and alpha-globulin was depressed.

Upon comparison with control animals, these results suggested that there is a much more pronounced reabsorption of albumins

and gamma-globulins than of either alpha-globulins or beta-globulins. While the D- and DL-serine produced the most marked variations from the controls, L-cysteine hydrochloride also increased urinary protein, but in the latter case, the normal distribution of proteins in the urine was found.

Hardy, Tantengco and Baumann have

clearly demonstrated the effect upon the rat of a massive distortion of the normal balance of amino acids in the diet, especially notable upon administration of a form of the amino acid (in this case D-serine) which is not readily metabolized by the animal. It will be interesting to see whether a similar alteration is produced in other species.

## EXPERIMENTAL ATHEROSCLEROSIS AND PYRIMIDINES

*Rats fed cholesterol and cholic acid were used in experiments comparing the potencies of uracil, thiouracil and related compounds in producing thyroid changes, hypercholesteremia, azotemia and the beginnings of atherosclerosis.*

Atherosclerosis in rats fed cholesterol and cholic acid is made worse by including thiouracil in the ration. In fact myocardial infarcts have been produced using this dietary modification. Naturally occurring pyrimidine derivatives have similar effects and uracil appears to be the most potent, according to L. C. Fillios, C. Naito, S. B. Andrus and A. M. Roach (*Am. J. Clin. Nutrition* 7, 70 (1959)).

Recently, Fillios, Naito, Andrus and Roach (*Circulation Res.* 8, 71 (1960)) compared the effects of uracil and thiouracil on experimental atherosclerosis in rats over a five-week period. Other related compounds were also investigated.

The atherogenic diet contained 20 per cent casein, 20 per cent hydrogenated cottonseed oil, 53.5 per cent sucrose, 1.5 per cent cholesterol, and 0.5 per cent cholic acid along with trace nutrients and salts.

In the first experiment, male rats averaging 260 g. were fed five different levels of thiouracil and compared with rats fed five different levels of uracil and with control rats fed only the atherogenic diet.

In the second experiment, males averaging 345 g. and females averaging 255 g. were compared. Each group was divided into subgroups fed either uracil, orotic acid, uracil plus orotic acid, thiouracil, or thiouracil plus orotic acid. Three other

groups of males received supplements of (a) thymine plus uracil, (b) urea, or (c) aspartic acid.

Determination of serum urea levels of animals fed the various diets showed that feeding of uracil and thiouracil results in progressive elevation of urea with time. Although pathologic changes such as calcification in renal tubules and bladder stone formation occurred in the urinary tract, they did not appear to be related to the variation in urea concentration. Serum urea level also did not correlate with the extent of cardiovascular lipid deposition.

Serum cholesterol averaged 278 mg. per cent in control animals after five weeks. The addition of 0.25 per cent of uracil to the ration brought the average serum cholesterol to 555 mg. per cent, and addition of 1.0 per cent uracil gave the maximum average cholesterol of 704 mg. per cent. Thiouracil at 0.125 per cent of the diet produced an average serum cholesterol response of 717 mg. per cent, and the average maximum of 1100 mg. per cent cholesterol was reached with 0.25 mg. per cent of thiouracil. The authors state that in experiments not yet published, uracil at 1 per cent of the diet doubled the serum cholesterol values even on rations lacking cholesterol and cholic acid.

In the second experiment, urea, aspartic acid or orotic acid supplementation caused



no striking elevation of serum cholesterol. However, addition of 1 per cent uracil to the ration produced a marked rise in serum cholesterol, averaging 660 mg. per cent in males. This rise could be prevented by combining uracil and orotic acid. Addition of thymine, however, did not reverse the uracil effect. When thiouracil was fed, the average serum cholesterol of males rose to 1131 mg. per cent, but in males fed both thiouracil and orotic acid the average was 631 mg. per cent.

Female rats were more susceptible to hypercholesteremia on the atherogenic diets than males, but inclusion of orotic acid eliminated the sex difference. In fact, females fed orotic acid and uracil averaged 154 mg. per cent serum cholesterol contrasted with 1193 mg. per cent from feeding the uracil supplement only. Thiouracil gave an average serum cholesterol of 1882 mg. per cent in females, but feeding both thiouracil and orotic acid resulted in an average of only 456 mg. per cent.

Cardiovascular sudanophilia increased in the uracil-fed animals as the level of serum cholesterol increased and as the level of dietary uracil increased. Endocardial sudanophilia was higher in the thiouracil-fed animals and was uniform regardless of the dietary level of thiouracil. The aortic and mitral valves and intervening endocardium were involved. Varying degrees of aortic sudanophilia were observed, which were difficult to quantitate but were correlated with increased endocardial sudan-

ophilia. None of the animals fed orotic acid showed any obvious aortic disease. The males supplemented with either urea or aspartic acid had less endocardial sudanophilia than the control animals.

Histologically, both uracil and thiouracil produce identical hyperplastic alteration of the thyroid differing only in degree. Comparisons of body weight, thyroid weight or serum cholesterol changes indicate that thiouracil is about 15 times as potent as uracil. Chemical analyses of the uracil certified its freedom from thiouracil contamination.

Hyperplasia of the thyroid was more marked in females fed uracil. On the other hand, females were more highly protected by the combined feeding of orotic acid and uracil as compared with males. Evidence of a protective action of orotic acid against thyroid hyperplasia due to feeding thiouracil was not definitive, either on the basis of organ weight or histological response. No definite histologic changes were seen when orotic acid was fed alone.

The goitrogenic action of uracil is unique among the naturally occurring pyrimidines. The compound could act in a manner similar to thiouracil or it could undergo conversion to a thiouracil-like compound in the organism. Orotic acid was used in this experiment because it is an intermediate in pyrimidine biosynthesis. Its action, according to the authors, might be related to its preferential position in the pathway of uridine-5-phosphate metabolism.

## SELENIUM AND VITAMIN E DEFICIENCY IN LAMBS

*Selenium administration protects against white muscle disease in vitamin E-deficient lambs and results in greater growth than does administration of vitamin E.*

Evidence is accumulating that selenium is an essential trace element in animal nutrition and that it and vitamin E have a mutually sparing effect in a number of experimental situations. The subject has been reviewed at length in this journal (*Nutrition*

*Reviews* 16, 149, 174, 319 (1958)). As pointed out, it previously had not been known that selenium was biologically essential except in the case of certain plants and in one bacterial enzyme system. However, work discussed in that review suggested that selenium is an

essential trace element in higher animals. At least two conditions were at that time preventable by either vitamin E or selenium: dietary liver necrosis in the rat and exudative diathesis (and "enlarged hock disorder") in the chick.

K. Schwarz, who has done considerable research on these dietary interrelationships, has recently reviewed "Factor 3, selenium and vitamin E" (*Nutrition Reviews* 18, 193 (1960)). He points out that selenium is an essential dietary constituent, and neither takes the place of vitamin E nor substitutes for it. He distinguishes diseases caused purely by vitamin E deficiency from others caused purely by Factor 3-selenium deficiency and still others caused by a simultaneous lack of both factors.

White muscle disease of lambs and calves evidently falls in the last group. Although there is evidence that this represents a vitamin E deficiency, selenium has also been reported to be protective (O. H. Muth *et al.*, *Am. J. Vet. Res.* 20, 231 (1959); (*Nutrition Reviews* 17, 171 (1959)).

In a more recent paper, J. E. Oldfield, Muth and J. R. Schubert (*Proc. Soc. Exp. Biol. Med.* 103, 799 (1960)) have shown that selenium or vitamin E is protective against white muscle disease of lambs and that selenium appears to be essential for growth.

The authors studied 48 pregnant ewes divided into four groups. The basal ration, presumably deficient in vitamin E and selenium, consisted of four pounds of Ladino clover hay and one-quarter pound of ground oats per head daily. The 20 lambs born of this control group showed an average six weeks' gain of 15.32 pounds; 16 of the 20 had gross lesions of white muscle disease. Ewes of lots two and three ate the same ration, but lot-two lambs were given 1.40 mg. selenium (as sodium selenite) intramuscularly (0.28 mg. at one day and 1.12 mg. at 14 days of age) while lot-three lambs received 1,000 IU vitamin E by mouth at one day and four days of age. Lot-four ewes were given the basal ration plus 0.1 p.p.m. selenium (as

sodium selenite). These diet treatments extended from the third month of gestation until the lambs were six weeks old.

All supplements protected completely against symptoms and gross lesions of white muscle disease in the lambs. However, pronounced growth differences were manifest. The six weeks' gain of the vitamin E-supplemented lambs (17.33 pounds) was not significantly greater than that of the unsupplemented group with white muscle disease (15.32 pounds). On the other hand, lambs given selenium averaged a gain of 20.84 pounds, while the increase in those from selenium-fed ewes averaged 27.15 pounds. The authors infer that selenium in the neonatal period, while correcting white muscle disease, is not available early enough to allow the full growth exhibited by lambs of selenium-treated dams. They conclude that these growth responses "appear to be further confirmation of the role of selenium as an essential nutrient, per se, necessary for normal growth even in absence of WMD [white muscle disease] lesions."

The authors do not discuss the content of selenium, vitamin E or oxidants and antioxidants in the ration they used. History warns of potential errors in interpretation in this area. In addition, the material was not studied microscopically, so that one cannot be sure that the muscle lesions were comparable in the various treated groups. Perhaps they differed in severity. Possibly varying the doses of vitamin E and selenium would show the apparent difference to be a dosage effect.

One would also like to know the effects on growth of selenium plus vitamin E at various dosages; are they additive or not? Further observations on this point might strengthen the conclusion that selenium is in itself essential for growth. This parameter of nutritional research is a very non-specific term to the experimental pathologist, to whom impaired growth encompasses most known diseases.

Oldfield, Muth and Schubert point out that selenium compounds have been inef-

fective in at least two vitamin E deficiency syndromes: muscular dystrophy in rabbits and fetal resorption in rats. Nonetheless, they have supplied one additional example

of the mutually sparing effect of selenium and vitamin E, while strengthening the opinion that their biologic effects are not identical.

## EFFECTS OF DIETS DEVOID OF VALINE AND LYSINE ON YOUNG RATS

*Force feeding of a diet devoid of valine and lysine produces a pathology in young rats similar to that of protein deficiency, with biochemical changes resembling those of kwashiorkor.*

The changes produced in human beings suffering from kwashiorkor indicate the urgent need for a better understanding of the mechanisms involved in the metabolism of dietary constituents, especially of protein and its amino acids. In a continuing series of experiments, H. Sidransky and co-workers have been investigating the morphological and biochemical changes occurring in young rats fed diets deficient in single amino acids. The very rapid development of changes in young rats force fed diets devoid of specific amino acids has provided a means of studying lesions which resemble those of kwashiorkor.

It has been particularly interesting that the changes resulting from feeding diets deficient in threonine, methionine and histidine have been remarkably similar (Sidransky and E. Farber, *Arch. Path.* **66**, 119, 135 (1958)). In an extension of this program, Sidransky and T. Baba (*J. Nutrition* **70**, 463 (1960)) have described the changes occurring in young rats fed diets devoid of valine and lysine. These changes resembled those resulting from diets deficient in threonine, methionine and histidine, and suggest that the deficiencies of many essential amino acids may produce changes which reflect an overall protein deficiency rather than a deficiency of a specific amino acid. For this reason tissue alterations differ very little.

On the other hand, dissimilar changes as a result of feeding diets deficient in individual amino acids suggest that some of the pathology which develops may be related to specific functions of individual amino acids. When

Sidransky and Baba force fed a diet devoid of valine or lysine to young rats weighing approximately 72 g. at the start of their experiments drastic changes were observed by the end of five or six days. A high rate of mortality in these rats led to an investigation of changes occurring by the end of three days. They concluded that many of the changes observed were well advanced by the end of three days of force feeding.

While rats on the control diet gained weight and showed no pathological changes, the animals fed the deficient diets lost weight even though the amounts fed were the same.

When fed ad libitum the valine- and lysine-devoid diets were poorly consumed and the animals lost weight, but they did not exhibit the changes observed in the animals force fed the diets devoid of valine or lysine.

The diet devoid of valine produced much more marked changes than that devoid of lysine. The hair of the valine-devoid rats soon became rough and shaggy and the animals grew listless and weak, with as many as 50 per cent dying after the first three days.

Of particular note were the liver changes. The total liver lipid content was significantly greater in the valine-devoid group than in the control group. The composition of the lipid was changed with a slight increase in cholesterol and a large increase in neutral fat. In the valine-devoid group there was also an increase in the liver glycogen content. Interestingly enough, the liver protein analysis was essentially the same for the amino acid-devoid groups and the controls.

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In the valine-devoid group there was a sharp decrease in the weight of the skeletal muscle (as exemplified by the gastrocnemius) accompanied by a decrease in protein content, but only a weight decrease in the lysine-devoid group.

These continuing investigations into the role of specific amino acids and the possible relationship of protein and amino acid deficiencies to biochemical changes in animals which resemble those occurring in kwashiorkor, have certainly demonstrated that a

deficiency of a single amino acid may be reflected as a protein deficiency with the major changes attributable to the low protein. Other biochemical changes, however, may be associated with the lack of a specific amino acid. By their force-feeding technique, these investigators have re-emphasized the role of amino acid balance and permitted an accentuation of morphologic and biochemical changes which may not appear with ad libitum feeding.

### VITAMIN K DEFICIENCY AND COPROPHAGY IN THE RAT

*Vitamin K deficiency, as manifested by increased prothrombin time, is uniformly induced in the rat by dietary deficiency of this vitamin if coprophagy is prevented.*

The failure to elicit manifestations of certain vitamin deficiencies in rats fed a synthetic diet deficient in that particular vitamin has been due, in many instances, to coprophagy since intestinal bacteria are capable of synthesizing many vitamins. The rat is quite resistant to the dietary induction of vitamin K deficiency, although occasional instances have been reported (J. D. Greaves, *Am. J. Physiol.* **125**, 429 (1939)). This resistance may be an effect of the great tendency of this animal to eat its feces. This possibility is explored in a recent report (R. H. Barnes and G. Fiala, *J. Nutrition* **68**, 603 (1959)).

These investigators fed male weanling rats a synthetic diet deficient in vitamin K and containing 1 per cent stripped corn oil as the sole source of fat. It was not possible to induce vitamin K deficiency with a diet containing 15 per cent hydrogenated vegetable oil, apparently because of the small amount of vitamin K contained in this preparation. Coprophagy was prevented by a small plastic cup covering the anus (Barnes *et al.*, *J. Nutrition* **63**, 489 (1957)). One-stage prothrombin times were determined on whole blood from the tail. Results of such determinations checked well with similar determina-

tions using oxalated plasma from heart blood.

Prothrombin times increased strikingly by the third to sixth weeks and then, peculiarly, decreased toward the normal level. Animals allowed access to their feces did not develop increased prothrombin times. Since several of the animals died during the period when the prothrombin time was greatly elevated, the possibility existed that the ultimate decline in prothrombin time was due to the selective survival of rats that showed little or no elevation.

The data were recalculated for only the animals which survived the vitamin K deficiency until the prothrombin time was again at a low level. Still the same typical curve with an elevation and a subsequent fall toward normal resulted, indicating that the terminal decrease was not due to selective survival of resistant animals. As the authors point out, this type of prothrombin time response seems to be typical of other vitamin K-deficient animals (T. D. Luckey, J. R. Pleasants and J. A. Reyniers, *J. Nutrition* **55**, 105 (1955)).

They also note that prothrombin time alone, as measured by the one-stage technique, does not measure the entire defect, since vitamin K deficiency decreases plasma

thromboplastin component (Factor IX) and proconvertin (Stable Factor) as well (R. L. Naeye, *Proc. Soc. Exp. Biol. Med.* **91**, 101 (1956); L. M. Fisher, G. J. Millar and L. B. Jacques, *Canad. J. Biochem. Physiol.* **34**, 1039 (1956)). Indeed, it has been questioned by some whether abnormalities in clotting mechanism alone could entirely explain the hemorrhagic phenomena which result (S. B. Wolbach and O. A. Bessey, *Physiol. Rev.* **22**, 233 (1942)). However, most of the animals that died did so during the period of greatest elevation of prothrombin time, although some died after it had decreased to a lower level.

The influence of vitamin K storage on the development of deficiency was studied by feeding animals the deficient diet while still allowing them access to their feces. At intervals up to 12 weeks, fecal collection cups were attached to groups of animals and prothrombin times determined. After three weeks on the deficient diet, there was an immediate increase in prothrombin time as soon as the animals were prevented from eating feces. However, during the first three weeks on the diet there was a lag period of one to two weeks after coprophagy was prevented before prothrombin times increased. This apparently indicates a storage of vitamin K, which is depleted after about three weeks on the deficient diet.

In another aspect of the study, two rats were caged together and fed the deficient diet. One was prevented from eating feces by the attachment of the plastic cup, whereas the second animal was allowed access to its own feces. Evidence of vitamin K deficiency, as manifested by an increased prothrombin time, occurred only in the animal with the plastic cup attached. However, this animal also probably managed to eat some feces from his cagemate since the prothrombin time increase was not as great as in control animals which were caged alone and which were prevented from eating feces.

Menadione in the diet, in amounts from 0.1 to 10.0 mg. per 100 g. of diet, prevented

the development of vitamin K deficiency whether or not coprophagy was prevented. As little as 1.0 mg. of either menadione or vitamin K<sub>1</sub> per 100 g. body weight given by stomach tube caused the prothrombin time to return to normal within 18 hours. Vitamin K<sub>1</sub> in doses of 0.5 mg. per 100 g. body weight had the same effect, but 0.1 mg. per 100 g. body weight was ineffective. It would seem that menadione is as effective as vitamin K<sub>1</sub> in reducing the prothrombin time in vitamin K-deficient rats, and that the minimal effective dose is somewhere between 0.1 and 0.5 mg. per 100 g. body weight.

There was a great variability between individual groups of animals in the spontaneous development of dietary vitamin K deficiency. Although only 19 animals developed signs of deficiency out of 84 rats fed the vitamin K-deficient diet but not prevented from eating feces, 16 of the 19 were from one study with 20 animals. In four similar experiments employing 39 rats no instance of deficiency was noted, and in two other groups with 25 rats three animals showed increased prothrombin times. The authors wisely conclude that the percentage incidence of the development of spontaneous hypoprothrombinemia would be of limited usefulness in view of this great variability. Much of this variability may be due to variations in the tendency of individual rats to eat feces.

It has been presumed that the relative difficulty in producing vitamin K deficiency in rats by dietary restriction is due to the synthesis of vitamin K by microorganisms in the intestine (*Nutrition Reviews* **3**, 35, 48 (1945)), and that the most effective means is to supplement the diet deficient in vitamin K with an antibiotic agent effective against intestinal bacteria (*Ibid.* **1**, 35 (1942); A. Kornberg, F. S. Daft and W. H. Sebrell, *Pub. Health Reports* **59**, 832 (1944)). *Escherichia coli* have been shown to possess a great ability to synthesize this vitamin, and the intestinal absorption of vitamin K produced by colon bacteria is thought to be sufficient

to supply the needs of most mammals (*Nutrition Reviews* 4, 324 (1946)).

However, the ability of the rat to absorb vitamin K from the intestine is questioned by the results reported in the present study. Indeed, the rat may have to eat its feces in order to get some of the vitamins synthesized in its intestine. The relationship of the rise and fall of the prothrombin time in vitamin K-deficient rats to intestinal synthesis and absorption is not known, although a similar curve has been found for vitamin K-deficient, germ-free chicks (Luckey, Pleasants and Reyniers, *loc. cit.*). A somewhat puzzling note is added by the report of the inability to produce dietary vitamin K deficiency in germ-free rats (Luckey *et al.*, *J. Nutrition* 57, 169 (1955)) and the conclusion that vitamin K is not required by the rat (*Nutrition Reviews* 14, 116 (1956)). It is possible that the diet used contained enough vitamin K to prevent induction of deficiency.

The ultimate mode of action of vitamin K is unknown at the present time. The factors in the clotting mechanism known to be affected by vitamin K deficiency are produced in the liver. Vitamin K may be concerned with the coupling of phosphorylation and oxidation in this organ (*Nutrition Reviews*

14, 211 (1956); J. P. Green, E. Sondergaard and H. Dam, *Biochem. Biophys. Acta* 19, 182 (1956)). Striking histologic abnormalities have not been noted in the liver or other organs except for hemorrhages (K. M. Endicott, Kornberg and Daft, *Pub. Health Reports* 59, 49 (1944); A. Ferraro and L. Roizin, *J. Neuropath. Exp. Neurol.* 2, 392 (1943)).

Electron microscopic examination of the liver and of capillaries in all parts of the body might be particularly informative; the former because it is the probable locus of action of vitamin K and the latter because alterations in capillary walls may be partially responsible for hemorrhagic phenomena.

This study re-emphasizes the fact that vitamin K is necessary in synthetic diets for the rat. Bacterial synthesis of vitamin K in the rat with absorption from the intestine is apparently not enough to meet the demands of the rat on a deficient diet. Coprophagy then enables the rat to utilize such material. This is a variable which must be considered in all studies attempting to induce dietary vitamin deficiency. This report affords another example of consistent dietary induction of a vitamin deficiency only after coprophagy is prevented.

## EFFECT OF INSULIN AND GLUCOSE ON TISSUE MAGNESIUM

*Administration of glucose and insulin to rabbits accelerates the uptake of radioactive magnesium<sup>28</sup> in many tissues and increases the magnesium content of skeletal muscle and heart.*

A number of intracellular constituents accumulate when glycogen is formed, either because of increased cell volume or because of chemical combination. Thus the administration of glucose and insulin, by increasing intracellular glycogen, causes substantial shifts of potassium, the principal intracellular cation, into the cell. The physiologic consequences of potassium derangements are particularly noticeable in cardiac and skeletal muscle where membrane electrical potentials or potassium content are signifi-

cantly altered, for example in hypokalemia and in familial periodic paralysis.

One might expect that the movement of other cell electrolytes would follow that of glycogen and potassium. In the case of magnesium, because of its largely intracellular position, one might anticipate physiological results of some consequence.

Recent developments in our knowledge of magnesium metabolism have been reviewed in this journal (*Nutrition Reviews* 18, 72, 101, 200 (1960)). Magnesium depletion



occurs in certain patients with alcoholism or electrolyte imbalance, especially in patients treated with magnesium-free parenteral fluids for long periods. The resulting syndrome is quite like that of low-calcium tetany. Because magnesium and potassium are both predominantly intracellular ions, there are a number of similarities in their metabolism.

J. K. Aikawa has studied the "Effect of glucose and insulin on magnesium metabolism in rabbits", using radioactive magnesium<sup>28</sup> (*Proc. Soc. Exp. Biol. Med.* **103**, 363 (1960)) and has found increased magnesium in heart and skeletal muscle.

Rabbits were fed a stock pellet ration. The author injected tracer doses of magnesium<sup>28</sup> in 1 to 2 mEq of stable magnesium chloride intravenously into each rabbit, followed by insulin, glucose, or both. Relative activity was calculated as counts per minute per gram of tissue divided by counts per minute per ml. of plasma and determined for various tissues, as was the magnesium content. The animals were sacrificed four hours after the first injection.

One group received only a tracer dose of magnesium<sup>28</sup>. The resulting specific activities were: bone, 12.9; kidney, 11.0; heart, 5.9; liver, 5.1; appendix, 3.9; skin, 1.25; muscle, 0.5. Another group received magnesium<sup>28</sup> plus subcutaneous insulin and dextrose. Apparently two different dosage combinations were used in each group and the results averaged. Relative magnesium activity increased significantly above the values in the control group for all tissues studied; it more than doubled in heart and skeletal muscle. Significant increases in magnesium content occurred in muscle (from 21.5 to 28.6 mEq per kg. of wet tissue) and in heart (from 13.0 to 23.5 mEq per kg.). The blood glucose value fell from 82.3 to 69.9 mg. per cent, but this was not considered significant (*P* was not <0.01).

When magnesium was followed by dextrose alone there was an increase in specific

activity of all tissues, considered significant only in the case of heart. Significant increases in magnesium content were found in both skeletal muscle and heart. Magnesium followed by insulin led to increased activity of all tissues, but the standard error of the mean was very large and the increases were not significant. There was a slight significant increase in heart magnesium content.

Aikawa points out that magnesium<sup>28</sup> is concentrated slowly in bone of man and dogs. In the rabbits, however, it was taken up most rapidly by bone, which had a magnesium content 13 times that of muscle, the tissue with the next highest content.

Although the dose of magnesium (1 to 2 mEq) was necessarily large because of the low specific activity of the material, serum magnesium levels were unchanged four hours after injection. Tissue levels were not compared but might well have also manifested little change.

The author mentions various reports relating magnesium, blood sugar and insulin and discusses the effect of magnesium on glycogenesis, on phosphatases and on adenosine triphosphate without arriving at a definite hypothesis. He concludes that insulin and glucose lead to deposition of magnesium in heart and skeletal muscle, and infers that "metabolism of magnesium is intimately related to that of carbohydrate and that the turnover of magnesium in tissues is regulated by insulin and dextrose."

The author's data leave some questions in the reader's mind. All three experimental groups consist of eight rabbits each, with four animals on each of two different dosage schemes. The results of the two subgroups are averaged in each group and the reader wonders why. The standard error of the mean for specific activities is as much as ten times higher in the magnesium plus insulin group as in the others; we are not told whether the variation in dosages or other factors are responsible for this. It is

difficult to appraise the method used for tissue magnesium determinations; it is defined only by a primary microfilm reference source. However, one infers that the method is satisfactory since the results are consistent with what one would expect from previous work on magnesium metabolism and similar work on potassium.

Tissue glycogen and potassium contents were not determined; it would be desirable

to compare these values with magnesium content.

In the absence of a detailed hypothesis of the interactions of magnesium, it seems simplest to explain Aikawa's results by suggesting that insulin and glucose cause increased intracellular glycogen deposition, increasing the fluid content of the cell and, with this, of the intracellular electrolytes including magnesium.

### VITAMIN B<sub>6</sub> DEFICIENCY AND PREGNANCY IN THE RAT

*Vitamin B<sub>6</sub> deficiency in the rat results in impaired production of red blood cells and hemoglobin. This defect is potentiated by pregnancy and may reflect a generalized defect in protein metabolism.*

Vitamin B<sub>6</sub> (pyridoxine, pyridoxal and pyridoxamine) has been implicated as being necessary in both fat metabolism, especially essential fatty acids, and in protein metabolism (*Nutrition Reviews* 12, 186 (1954)). As a precursor to pyridoxal phosphate it functions as a coenzyme in several transaminating and decarboxylating reactions. An adequate supply of this vitamin is necessary for maintenance of normal protein metabolism and growth. Thus the effects of deficiency should be especially acute during periods of maximal protein demand for tissue growth, such as during pregnancy. Alterations in plasma protein, hemoglobin, and hematocrit in the pregnant rat have recently been reported (R.L. Pike and M.L. Brown., *J. Nutrition* 68, 551 (1959)).

These investigators placed female rats weighing about 200 g. in eight groups. One group of nonpregnant rats was fed an adequate basal synthetic diet supplemented with 0.8 mg. pyridoxine per 100 g. diet and another nonpregnant group was fed the basal diet containing 4 mg. deoxypyridoxine per 100 g. diet. Two groups of pregnant rats were fed similar diets. Other animals were fed the deoxypyridoxine-supplemented diet for one to four weeks prior to mating to test the effect of depletion of vitamin B<sub>6</sub> stores on pregnancy. Subsequent to mating,

one group of the latter animals was continued on the deoxypyridoxine-containing diet while the remainder were fed the diet supplemented with pyridoxine.

Nonpregnant controls for the latter two groups underwent a uniform depletion period of 21 days and were then divided into two groups, one of which was continued on the vitamin B<sub>6</sub>-deficient diet and the other fed the vitamin B<sub>6</sub>-supplemented diet. Blood was collected on the first, eighth, fifteenth, and twenty-first days of pregnancy. Specific gravities of whole blood and plasma were determined. Hematocrit, hemoglobin, and plasma protein levels were calculated from the specific gravity values.

During a three-week period, vitamin B<sub>6</sub> deficiency in nonpregnant animals resulted in a steadily increasing plasma protein level (a total increase of about 7 per cent of the initial value) and an early rise in hemoglobin and hematocrit with a subsequent plateau when compared to the nonpregnant animals supplemented with vitamin B<sub>6</sub>. A somewhat similar response occurred in the deficient and supplemented pregnant animals during the first two weeks. During this period the plasma protein level in the deficient animals rose slightly, but then fell precipitously during the third week (a total decrease of about 11 per cent of the

initial value), as it did in the pregnant supplemented group.

Prior vitamin B<sub>6</sub> depletion prevented pregnancies from reaching term in those animals continued on the deoxypyridoxine-supplemented diet; in half of the group implantation did not occur and in the other half the fetuses were resorbed prior to the third week. In the depleted group fed a pyridoxine-supplemented diet after mating, the response was closely similar to that in non-depleted, non-deficient pregnant animals. Rats continued on the deficient diet (no pregnancies reaching term) showed a response intermediate between pregnant and non-pregnant deficient animals.

Pregnancy affected both depleted and non-depleted, supplemented and deficient animals in similar manner. There was a steady decline in these values during the entire three weeks of pregnancy (a total decrease of about 20 per cent of the initial value). However, the decrease in these values in pregnant deficient animals (both depleted and non-depleted) occurred earlier and was quantitatively greater than that in the pregnant, supplemented animals. Since nonpregnant, deficient animals showed a similar response, it seemed that the diet and pregnancy had a synergistic action in lowering the hemoglobin and hematocrit levels.

The authors believe that these changes in plasma protein, hematocrit, and hemoglobin levels reflect altered ability of the organism to form plasma proteins, red blood cells and hemoglobin. However, as they point out, many of the changes can be explained on the basis of alterations in blood volume that are known to occur as the result of pregnancy and of vitamin B<sub>6</sub> deficiency (Brown and Pike, *Fed. Proc.* 17, 472 (1958)). Hemoconcentration occurs in deficient, nonpregnant animals. Hemodilution occurs in pregnant animals but is less marked in deficient than in supplemented animals. Thus the increases in total plasma protein and hematocrit and hemoglobin in the

deficient, nonpregnant animals probably reflect a reduced blood volume.

Changes in plasma protein in the pregnant, deficient and supplemented rats also probably reflect to a large extent changes in blood volume. This is consistent with the findings of others that vitamin B<sub>6</sub> deficiency does not affect the level of serum proteins or blood amino nitrogen (J. R. Beaton *et al.*, *J. Biol. Chem.* 200, 715 (1953)).

However, continued deficiency apparently results in a decrease in hemoglobin and hematocrit, which is not explicable on the basis of blood volume changes and which is potentiated by pregnancy. The authors believe that this indicates a loss of ability to synthesize hemoglobin and red blood cells. To corroborate this they have shown, as have other workers (A. Kornberg, H. Tabor and W. H. Sebrell, *Am. J. Physiol.* 143, 434 (1945); W. W. Hawkins, B. Lechow, and M. K. Evans, *Ibid.* 170, 155 (1952)), that the deficient animal is unable to adequately replace red cells lost by removal of blood.

Red blood cell counts and appearance in smears were not reported and, therefore, although hemoglobin and hematocrit levels are known, it is not possible to say whether the altered production of cells is reflected in cells of abnormal size and/or abnormal hemoglobin content. Such values would be desirable and are of such simplicity that they should be included in all studies concerning the red cell.

Others (J. M. Batchen *et al.*, *Brit. J. Nutrition* 2, 14 (1948)) have reported that vitamin B<sub>6</sub> deficiency in the rat results in increased red cell count, decreased hemoglobin, reduction in mean corpuscular volume, and reduction in mean corpuscular hemoglobin concentration; that is, a microcytic, hypochromic anemia. Increased numbers of normoblasts also appear. Thus it appears that vitamin B<sub>6</sub> deficiency causes impaired production of normal erythrocytes and this effect may be further potentiated by the demands of pregnancy.

The pathogenesis of the anemia of vitamin B<sub>6</sub> deficiency is not known and future studies might well pursue this objective. Occurring concomitantly with the microcytic, hypochromic anemia is a progressive hypersideremia and storage of iron in tissues as hemosiderin (*Nutrition Reviews* 3, 29, 343 (1945)). There is no evidence of hemolysis and the primary abnormality appears to be a defect in heme synthesis (M. P. Schulman and D. A. Reichert, *J. Biol. Chem.* 226, 181 (1957)), perhaps in the formation of protoporphyrin (G. E. Cartwright and M. M. Wintrobe, *Ibid.* 172, 557 (1948)).

The reason for the development of increased plasma iron is apparently a normal or increased absorption of iron combined with a decreased iron utilization in the formation of hemoglobin. If dietary iron is concomitantly restricted, hyperferremia does not develop (*Nutrition Reviews* 3, 269 (1945); 8, 5 (1950)). Histologic studies of bone marrow in the rat have not been found, but in the pig and dog, in which species the anemia is more severe, there is a marked hyperplasia of erythropoietic elements (*Ibid.* 3, 29, 343 (1945)).

It is interesting that several cases of hypochromic, hypersideremic anemia in man which respond favorably to vitamin B<sub>6</sub> therapy have recently been reported (G. Gehrman, *Folia Haemat.* 2, 225 (1958); S. E. Snyderman *et al.*, *Am. J. Clin. Nutrition* 1, 200 (1953); J. W. Harris *et al.*, *Proc. Soc. Exp. Biol. Med.* 91, 427 (1956)). The relation of this to the hypochromic, hypersideremic anemia in man in which a morphological abnormality of iron accumulation in mitochondria of immature red cells has recently been observed (A. Policard and M. Bessis, *Comptes Rendus Acad. Sci.* 246, 3194 (1958)) is unknown, but it is probable that study by electron microscopy of erythropoietic tissue in vitamin B<sub>6</sub>-deficient experimental animals would give valuable information.

The effect of maternal deficiency on the developing embryo is of great interest and is only briefly mentioned in the studies reported here. Marked reproductive upsets in rats depleted of vitamin B<sub>6</sub> prior to pregnancy and continued on a deficient diet during pregnancy have been reported (*Nutrition Reviews* 15, 20 (1957)). These reproductive derangements have consisted of fetal resorptions, failure of implantation and underweight fetuses. No congenital malformations have been reported.

The effect of pyridoxine deficiency on pregnancy is similar to the effect of deficiencies of several other vitamins and essential food factors (M. M. Nelson and H. M. Evans, *J. Nutrition* 31, 497 (1946)), including deficiency of essential fatty acids (G. O. Burr and M. M. Burr, *J. Biol. Chem.* 86, 587 (1930)). The latter relationship is of particular interest because of the apparent importance of vitamin B<sub>6</sub> in essential fatty acid metabolism. It would be of interest to know whether supplementation of the vitamin B<sub>6</sub>-deficient diet with a superabundance of essential fatty acids would partially or completely inhibit the effect of vitamin B<sub>6</sub> deficiency on reproduction.

The defects in protein metabolism occurring in vitamin B<sub>6</sub> deficiency are probably responsible for the aberrations in red cell production and fetal development as well as for other abnormalities. The ultimate defect appears to be an abnormality in the metabolism of amino acids so that there is increased amino acid catabolism (E. W. McHenry, in *Current Research of Vitamins in Trophology*, p. 21. National Vitamin Foundation, Inc., New York (1953)). Observations consistent with this viewpoint are decreased nitrogen retention (Beaton *et al.*, *loc. cit.*), elevated fasting blood urea (Hawkins, M. L. McFarland, and McHenry, *J. Biol. Chem.* 166, 223 (1946)), increased rate of urea formation in liver slices from deficient rats (E. F. Caldwell and McHenry, *Arch.*

*Biochem. Biophys.* **48**, 50 (1954)), and aggravation of vitamin B<sub>6</sub> deficiency by high protein diets (E. C. Miller and C. A.

Baumann, *J. Biol. Chem.* **157**, 551 (1945)) and by growth hormone (J. L. Beare *et al.*, *Endocrinology* **55**, 40 (1954)).

### CECAL ENLARGEMENT IN GERM-FREE ANIMALS

*Germ-free animals have ceca which, with their contents, weigh about six times that of conventional animals. This increase in size is apparent in rats by the second week of life.*

One of the major difficulties confronting workers in the germ-free area is the development of an enlarged cecum in all species of animals successfully raised in the germ-free state. This situation was recognized as early as 1896 by G. H. F. Nuttall and H. Thierfelder (*Ztschr. Physiol. Chem.* **22**, 62 (1896)), who reported that at two weeks of age their germ-free guinea pigs had enlarged ceca filled with a fluid-like material.

B. Wostmann and E. Bruckner-Kardoss (*Am. J. Physiol.* **197**, 1345 (1959)) reported that rats and mice raised in the germ-free state could successfully reproduce and rear young even though their ceca were approximately five times normal size. A similar situation in the rabbit or guinea pig, however, appears to have an adverse influence on reproduction. The above investigators report that there has been only one litter of germ-free guinea pigs born to a germ-free mother.

The relationship of the enlarged cecum to reproductive failure is unknown. It has been observed that viable spermatozoa could be secured from epididymus and ductus deferens of male guinea pigs (B. Philips, P. Wolfe and H. A. Gordon, *Ann. N. Y. Acad. Sci.* **78**, 183 (1959)). The ovaries in the females showed no evidence of ovulation or of corpus luteal tissue.

In germ-free rabbits the cecum and its contents may sometimes represent one-third of the body weight. This enlarged cecum sometimes gets twisted, thus preventing cecal flow. This has been the only apparent cause of death in a number of animals (Wostmann and Bruckner-Kardoss, *loc. cit.*).

Wostmann and Bruckner-Kardoss (*loc. cit.*) measured the size of ceca in germ-free rats. They hoped to determine whether the cecal enlargement was due to a deficiency or imbalance of nutrients in the sterilized diets or to the absence of microbes in the intestinal tract. Twenty-one germ-free animals were permitted nothing but milk from the time they were born. After the first ten days they were separated from their dam but every four hours the mother was placed in their cage for a two-hour nursing period. By this means the young were kept away from solid food. Another 21 germ-free animals were permitted access to a sterilized semi-purified diet in addition to the mother's milk. Conventional control animals were reared in a regular animal colony.

At the seventh day of life one-third of the animals in each group was sacrificed, but no difference was found in the body weights of the animals nor in the size of their ceca. However, by the sixteenth day the seven germ-free rats with access to the diet weighed 23 g. and had ceca averaging 1.6 g., while the conventional rats weighed 33 g. with ceca weighing 0.4 g. The germ-free rats receiving nothing but mother's milk weighed 20 g. and had ceca weighing 0.9 g., while the conventional control rats receiving nothing but mother's milk weighed 24 g. and had ceca weighing 0.3 g. Similar differences between the germ-free and the conventional animals were apparent at the twenty-fifth day, at which time the weights of the ceca of the germ-free animals were from 5.5 to 6.4 times those in the conventional rats.

Five male and four female germ-free rats were continued on the sterile semi-purified diet until they were 42 days of age. At this time they weighed 94 and 74 g., respectively, while the conventional animals weighed 159 and 119 g., respectively. The weight of the ceca in the germ-free animals was from 6.6 to 7.4 times that in the conventional animals. Similar results were secured using a sterilized "practical type" diet.

The investigators assumed that mother's milk is a fairly complete food. Thus when the animals receiving nothing but mother's milk showed enlargement of the ceca, they suggested that this enlargement was due to some factor other than nutritional deficiency.

In the present study, the germ-free rats did not grow as rapidly as the conventional animals (94 g. vs. 159 g. for males at 42 days of age). This was contrary however, to earlier observations on chicks by the same authors (*Nutrition Reviews* 17, 236 (1959)). Thus until further study has been made of the poor growth of the germ-free rat, it may be premature to conclude that the enlarged ceca is not due to a nutritional deficiency.

The incidental observations made in conjunction with the above study indicated that the water content of the small intestine was the same in the germ-free and the

conventional rats (76 vs. 74 per cent). On the other hand, the ceca of the germ-free rats contained considerably more water than in the conventional animals (84 vs. 74 per cent). To determine whether the bacterial flora in the conventional rats might bind minerals and thus reduce the osmotic pressure, Westmann and Bruckner-Kardoss fed germ-free rats a purified diet containing a very low salt content. This, however, had no effect on the enlargement of the ceca.

They also observed that the histamine concentration in the cecal contents of germ-free guinea pigs was 2.5  $\mu$ g. per g. whereas in the conventional animals it was 10.3  $\mu$ g. per g. Rats showed similar but smaller differences. The authors were unable to increase the histamine level in the ceca of germ-free rats by anal catheterization.

The above observations are intriguing in that they show another marked difference in the physiology and anatomy of the germ-free and conventional animal. One might hope that subsequent work will utilize a sterile extract of the intestinal contents of conventional rats. If this or a sonic preparation of the intestinal contents were fed to germ-free animals, it should be possible to determine whether bacterial products are responsible for the smaller ceca in the conventional animals.

## EXPOSURE OF NIACIN AND AMINO ACIDS TO ETHYLENE OXIDE

*Exposure of certain nutrients to ethylene oxide results in hydroxyethylation of tertiary nitrogen atoms and of sulfur atoms with consequent loss of biological activity.*

A decrease in the nutritional value of food exposed to the fumigant, ethylene oxide, was demonstrated a few years ago by E. A. Hawk and O. Mickelsen (*Science* 121, 442 (1955); *Nutrition Reviews* 14, 55 (1956)). These workers established that thiamine was one of the nutrients adversely affected by ethylene oxide, but concluded that other factors must have been altered as well.

Since then, treatment with ethylene oxide

has been shown to cause at least partial destruction of the following vitamins: riboflavin, niacin, pyridoxine, and folacin (H. Bakerman *et al.*, *J. Agr. Food Chem.* 4, 956 (1956)); and the following amino acids: histidine, methionine (H. G. Windmueller, C. J. Ackerman and R. W. Engel, *J. Nutrition* 60, 527 (1956)) and, most recently, lysine (Windmueller, Ackerman and Engel, *J. Biol. Chem.* 234, 895 (1959)). The general



problem of chemicals and food processing has been reviewed by Mickelsen (*Nutrition Reviews* 15, 129 (1957)).

Until recently, the nature of the reaction of ethylene oxide with these nutrients had not been elucidated. However, Windmueller, Ackerman, Bakerman and Mickelsen (*J. Biol. Chem.* 234, 889 (1959)) have now identified reaction products of ethylene oxide with niacinamide and with niacin, and Windmueller, Ackerman and Engel. (*Ibid.* 234, 895 (1959)) have strong evidence of the nature of the reaction products with histidine, methionine and cysteine.

In the first study, a solution of the pure nutrient in a previously evacuated vessel was exposed to ethylene oxide gas at approximately atmospheric pressure. Facilities were available to measure and regulate pH during the exposure, since at times reaction products were strongly alkaline. It was properly realized that the conditions used did not duplicate commercial methods of ethylene oxide sterilization of foods. However, the objective was to produce large quantities of reaction products for identification.

When the pH of the reaction mixture of niacinamide and ethylene oxide was maintained between 7 and 10.5 by adding hydrochloric acid, a product was obtained in crystalline form, which was identified as the chloride of N'-(2-hydroxyethyl) niacinamide. In other words, the hydroxyethyl group had added to the tertiary ring nitrogen of niacinamide. Since this product fluoresced when subjected to the assay procedure of J. W. Huff and W. A. Perlzweig (*J. Biol. Chem.* 167, 157 (1947)) for N'-methylnicotinamide, a fluorometric assay was used to quantitatively determine the product. The N'-(2-hydroxyethyl) nicotinamide was unstable at pH 11 to 12, producing a variety of reaction products, and this explained why it was not possible to identify this reaction product unless the highly alkaline conditions were avoided.

The authors present evidence that this same compound is probably produced in foods treated with ethylene oxide. N'-(2-hydroxyethyl) nicotinamide neither replaced nor was a metabolic antagonist for niacin as judged by the response of the microorganism, *Lactobacillus arabinosus*, or of chicks maintained on low-niacin regimens.

A similar reaction occurred between ethylene oxide and niacin, and the product was identified as the internal salt, the betaine of N'-(2-hydroxyethyl) niacin. At pH 6 or above, the biological activity of niacin was destroyed to a greater extent than that of niacinamide. The destruction of both compounds by ethylene oxide was highly dependent on temperature and was more extensive at pH values above neutrality.

Of interest was the discovery that DPN, a coenzyme form of niacin, was completely stable to ethylene oxide treatment as determined by microbiological assay. This fact, along with variation in moisture, pH and reaction temperature, probably largely accounts for the marked variations in susceptibility to ethylene oxide fumigation of niacin activity in various food products. For example, under standardized conditions of fumigation, only 22 per cent of the niacin activity was destroyed in brewers' yeast compared to 84 per cent in enriched white flour.

Windmueller and co-workers point out that each of the vitamins shown to be labile to ethylene oxide treatment contains a tertiary heterocyclic nitrogen, and, although direct evidence is lacking, the authors suggest that this site is most likely the point of attack by ethylene oxide.

In the second paper, Windmueller *et al.* (*J. Biol. Chem.* 234, 895 (1959)) present results of a study of the reaction products of ethylene oxide with proteins and with certain amino acids. While previous work had shown that casein treated with ethylene oxide exhibited a lowered biological availability of histidine and methionine, the recent

work indicates that lysine is also affected and that lactalbumin and egg albumin behave similarly to casein.

In this study, imidazole was used as a model compound and comparisons were made of the reaction of ethylene oxide with histidine and with imidazole. From the results it was concluded that ethylene oxide caused hydroxyethylation of both nitrogen atoms of the imidazole ring. Destruction of histidine in intact protein (casein) exposed to ethylene oxide was demonstrated by chemical assay for histidine and was shown to be increased markedly if the protein contained moisture. However, in certain proteins (chymotrypsin) the histidine moieties appeared not to react unless the protein was denatured in 8 molar urea.

Chemical studies of the reaction products of ethylene oxide with methionine and with N-acetylmethionine indicated that one hydroxyethyl group had added to the sulfur

atom of these compounds to produce a sulfonium group. A similar double alkylation of the mercapto group of cysteine occurred and with this amino acid the primary amino group also became alkylated. Although the reaction product with lysine was not identified, it was assumed that hydroxyethylation of one or both of the primary amino groups of lysine could account for the decreased availability of this amino acid.

The authors generalize by stating that the reactions of these nutrients with ethylene oxide under mild conditions involve electrophilic hydroxyethylation of an atom with one or more lone pairs of electrons, either nitrogen or sulfur. In no case has it been shown that these hydroxyethylated derivatives possess the biological activity of the original nutrient. These studies amply clarify in a qualitative manner how ethylene oxide, as a commercial fumigant, may lower the nutritive value of foods.

### ENHANCEMENT OF CALCIUM ABSORPTION BY CARBOHYDRATES

*Studies of  $\text{Ca}^{45}$  absorption from the upper and lower small bowel of rats indicate that several sugars enhance calcium absorption in the ileum. Simple sugars are absorbed too rapidly to contribute to this effect.*

In economically depressed countries, the diet of most persons is composed more of vegetable foods than of animal foods. Usually these circumstances result in a lower intake of calcium than generally considered optimal. Nevertheless, A. R. P. Walker *Am. J. Clin. Nutrition* **3**, 114 (1955) reported that children in several such countries did not have a high incidence of rickets, nor was their rate of growth benefited by the addition of calcium to their diets. A similar report (*Nutrition Reviews* **13**, 131 (1955)) gave evidence that children who had eaten a diet composed largely of cereals apparently were not deficient in calcium since the addition of milk or of fortified flour to their diets failed to improve their growth. Either our estimates for calcium requirement have been too high, or these children

have been able to use calcium more economically than children fed the usual American diet.

For many years it has been recognized that dietary lactose facilitates absorption of calcium from the gut. This subject was reviewed recently (*Nutrition Reviews* **18**, 115 (1960)) and evidence was presented to show that lactose has a direct effect on the intestinal wall.

O. W. Vaughan and L. J. Filer, Jr., (*J. Nutrition* **71**, 10 (1960)) studied this problem in further detail. They considered several theories: (1) that lactose lowers the pH of the gut by promoting fermentation, (2) that some sugars influence the bone cells in the process of ossification, (3) that lactose (as well as other sugars) may cause stimulation of the flow of digestive juices, including

bile, which in turn may augment calcium absorption.

The authors employed  $\text{Ca}^{45}$  to study the rate of absorption from isolated loops of bowel in rats. Loops were isolated in the proximal small bowel (duodenum) or in the distal small bowel (ileum). Test solutions containing one of the sugars and calcium chloride tagged with  $\text{Ca}^{45}$  were injected into these intestinal loops. The effects of bile and of other ions ( $\text{Mg}^{++}$ ,  $\text{Sr}^{++}$ ,  $\text{Na}^+$ ) were similarly studied. After four hours, the animals were sacrificed and their femurs ashed for determination of  $\text{Ca}^{45}$  content. In some of the studies, the intestinal loops also were removed to determine the amount of  $\text{Ca}^{45}$  remaining unabsorbed. The sugars employed were lactose, sorbitol, glucose, sucrose, fructose, galactose and xylose.

None of the substances had a significant enhancing effect on the rate of calcium absorption from the duodenum. However, in the ileum each of the sugars enhanced the rate of  $\text{Ca}^{45}$  absorption. The addition of  $\text{Mg}^{++}$  or  $\text{Sr}^{++}$  to the test solution prevented

the enhancing effect of sugars, but  $\text{Na}^+$  did not. Addition of phosphate inhibited calcium absorption both alone and with sugar.

It is apparent that most of the calcium was absorbed from the distal portion of the bowel. Although all of the sugars tested could enhance this rate of absorption, simple sugars would not reach this level but would be absorbed in the upper bowel. Indeed, it has been shown that feeding rapidly absorbed sugars with a test dose of calcium had no effect. However, the more complex carbohydrates, which are absorbed lower in the digestive tract, did enhance absorption of calcium (R. H. Wasserman and C. L. Comar, *Proc. Soc. Exp. Biol. Med.* **101**, 314 (1959)).

This may explain in part the ability of children eating crude vegetable diets to absorb a quantity of calcium sufficient for growth, even though the supply is meager. In all probability other factors may be involved, but complex carbohydrates are undoubtedly of assistance.

### CHANGES OCCURRING WITH VITAMIN A DEFICIENCY

*With increasing duration of vitamin A deficiency in calves, cerebrospinal pressure increases as a result of increased volume. A decrease in potassium level of aqueous humor also occurs.*

In contrast with the definite changes in epithelial tissues, bones and teeth, which occur in avitaminosis A, the metabolic role of vitamin A aside from its function in vision remains obscure. Observations of L. A. Moore and J. F. Sykes (*Am. J. Physiol.* **130**, 684 (1940)) that cerebrospinal fluid pressure increased in vitamin A-deficient calves soon led to similar observations in other species. Furthermore, the similarity of the mechanism whereby cerebrospinal fluid and aqueous humor are formed (H. Davson, *Physiology of Ocular and Cerebrospinal Fluids*. Little Brown and Co., Boston (1956)) has raised the suggestion that intra-

ocular pressure may increase in vitamin A deficiency.

Some of these considerations led B. A. Dehority *et al.* (*J. Dairy Sci.* **43**, 630 (1960)) to study the changes occurring in the composition of cerebrospinal fluid and aqueous humor with increasing duration of vitamin A deficiency in an attempt to relate these changes to the increased pressures of these fluids in the deficient animal.

Dehority and co-workers used male Holstein calves, which they depleted to a plasma vitamin A value of 8.3  $\mu\text{g}$ . per 100 ml. of plasma and then supplemented with carotene (supplied by alfalfa) at levels of 16, 24, 32 and 40  $\mu\text{g}$ . per pound of live weight

per day. The lower two levels were designed to provide marked increases in cerebrospinal fluid pressure, the 32  $\mu\text{g.}$  level to produce moderate increases, and the 40  $\mu\text{g.}$  level to produce no increases in the pressure of this fluid. The calves were maintained on these levels of carotene intake for a period of 16 weeks, at which time the animals were sacrificed and extensive analyses carried out on various tissues and fluids of the body. In addition to the analyses, cerebrospinal fluid pressures were measured ten and three days prior to sacrifice. Intraocular pressures were measured before carotene supplementation was begun and five days prior to sacrifice.

Cerebrospinal pressures increased from 44 ml. of saline with the carotene intake of 40  $\mu\text{g.}$  per pound of live weight per day to 175 ml. with the 24  $\mu\text{g.}$  intake. Pressure with the 32  $\mu\text{g.}$  intake was 131 ml. In an effort to determine the mechanisms whereby this pressure was caused, these investigators point out that in the vitamin A deficiency pattern where tissue changes were not apparent, chemical changes in blood serum, cerebrospinal fluid and aqueous humor were for the most part nonsignificant. Plasma vitamin A showed increases with increasing levels of carotene intake and there was also an increase in carotenoid and vitamin A content of the liver with increasing levels of carotene.

The most outstanding observation was the very little change in composition of the cerebrospinal fluid. Thus these investigators

concluded that the pressure associated with vitamin A deficiency is due to an increased volume of cerebrospinal fluid caused either by increased formation of the fluid or by decreased absorption, or possibly both. An evaluation of the aqueous humor showed that the slight increase in intraocular pressure was not significant and it was concluded that either the method of measurement was of insufficient accuracy or that there were factors other than vitamin A deficiency controlling the regulation of intraocular pressure.

In the chemical evaluation of the constituents, it was found that the potassium level of aqueous humor showed a significant decrease with the lower intakes of carotene. This was accompanied by an increase in chloride content with a slight net increase in osmotic pressure.

The investigations of Dehority and co-workers are of interest since they emphasize the very slight alterations in the chemical composition of fluids which nonetheless exhibit changes in volume and, in the case of the cerebrospinal fluid, changes in pressure. The changes occurring in the aqueous humor certainly are of interest and point to the need for further study of such alterations under more severe conditions of vitamin A deficiency. The very suggestive work of these investigators points up the need for continued study of the locations of cerebrospinal fluid absorption and formation as possible areas where vitamin A exerts a specific metabolic function.

## NOTES

## Site of Fatty Acid Absorption

The intestinal absorption of fat has been investigated so frequently during the past two decades that at times it has seemed that little could be gained from its further study. Nevertheless, although the mechanism of absorption in rats is now fairly well known, it is probable that species differences exist. Moreover, certain other details remain to be worked out, such as the site of absorption.

In order to clarify this phase of fat absorption, J. M. Johnson (*Proc. Soc. Exp. Biol. Med.* **100**, 669 (1959)) made use of a technique which he had developed previously (*Ibid.* **98**, 839 (1958)) to study fatty acid absorption. Small everted sacs were prepared from successive segments of the small intestines of hamsters. These were filled with bicarbonate buffer and glucose solution and were immersed in solutions of palmitic-1-C<sup>14</sup> acid-albumin complex also containing glucose and were incubated with 95 per cent oxygen and 5 per cent carbon dioxide at 37°C. Aliquots of serosal and mucosal solutions and homogenized intestinal wall were plated for counting and the lipids were separated into free and esterified fatty acids.

The results of these analyses revealed that activity in the serosal solutions and intestinal walls was greatest for the uppermost jejunal segment and decreased progressively with each lower segment. The reverse was true for the mucosal solutions. This information was strengthened by the finding that, generally, the ratios of activity of the esterified to free fatty acids of the mucosal portions were greater in the upper than in the lower segments. In the serosal solutions, the activity of the esterified portion of the fatty acids was greater than 90 per cent of the total.

These results indicate a considerably greater ability of the upper portion of the

hamster intestine to absorb and esterify fatty acids. The actual site of maximum absorption, however, may depend partially on other factors such as size of fat meal, intestinal motility and state of emulsification. Thus, J. A. Benson, G. N. Chandler, F. E. Vansteenhuyse and J. O. Gagnon (*Gastroenterology* **30**, 53 (1956); *Nutrition Reviews* **15**, 93 (1957)) reported that I<sup>31</sup>-labeled olive oil is absorbed maximally in the third quarter of the small intestine of the rat. If rats and hamsters can be compared and if iodinated olive oil and fatty acid-albumin complex are absorbed by the same mechanism, their reasoning that this portion of the intestine has "a heightened capacity for cell work, perhaps by cellular enzyme systems," is not supported by the experiments of Johnson.

It seems evident from these considerations that even in the case of this relatively simple phase of fat absorption, considerable work remains to be done.

Extreme Hypcholesterolemia  
with Steatorrhea

In the voluminous literature on cholesterol metabolism, much of it relating to hypercholesterolemia and its presumed causes and effects, there are far-reaching assumptions as to the value of reducing serum cholesterol levels in the prevention of atherosclerosis. Scarcely anything is written, however, about hypocholesterolemia.

I. S. Friedman, H. Cohn, M. Zymaris and M. G. Goldner have reported a case of lifelong "Hypocholesteremia in idiopathic steatorrhea" under this title (*A. M. A. Arch. Int. Med.* **105**, 112 (1960)). Their patient, a 36-year-old white man, has been confined to a hospital for chronic diseases for over 20 years; throughout this period his serum total cholesterol concentration has ranged between 25 and 45 mg. per cent.

The clinical history and findings are exceedingly complex. Celiac disease was diag-

nosed at 18 months and treated with a banana and meat diet for years. When 14 years old, the blood serum cholesterol level was found to be 42 mg. per cent. At 36 years of age the patient is malnourished. In the laboratory tests performed over the years, occasional traces of protein were found in the urine. The hemoglobin remained near 14 g. per cent, red blood cells over 4 million per cubic millimeter, plasma albumin 4.3 g. per cent, and globulin 2.1. Cephalin flocculation at times varied from + to +++; serum amylase was somewhat depressed (29 to 53 units per cent).

The outstanding chemical abnormalities were in the serum lipids, which totaled only 110 mg. per cent; of this, total cholesterol was 25 mg. per cent (7 free, 18 esterified). Vitamin A absorption was close to zero. Glucose tolerance was normal. Creatinine excretion was above normal, probably because of muscle wasting. The 24-hour urinary excretion of 17-ketosteroids was very low (5.5 mg.) and there was no increase after corticotrophin injection. Both fat digestion and absorption were shown to be impaired.

Normal thyroid and liver function tests exonerated these organs. However, the low urinary excretion of 17-ketosteroids and its failure to rise after corticotrophin injection suggested hypofunction of the adrenal cortex as a factor in the patient's low serum lipid values.

The infantile sprue and later steatorrhea in this case surely represent one entity, and the authors' diagnosis is nontropical sprue or adult celiac disease, in spite of the normal

glucose tolerance, lack of anemia and normal serum calcium. The patient's diet is self-selected: 2400 calories, high in carbohydrate, with much bread, moderate protein and little fat. He has refused a gluten-free diet. A number of physical findings suggest genetic disease.

Because of the normal cholesterol and phospholipid content of the stools, Friedman *et al.* infer that malabsorption of these substances is not responsible for their low serum levels. The evidence does not establish that the patient has either decreased formation or increased degradation of his serum lipids.

As to the diminished adrenal cortical function, one is unable to relate it in detail to the other findings inasmuch as hypocholesterolemia may be both cause and effect of decreased steroid output. One can believe, with the authors, that the adrenal cortical deficiency is the result of malnutrition, but this does not say much as to the relationship between adrenal steroids, lipid absorption and serum lipid levels. Established vitamin deficiencies in this patient complicate matters still further.

Although moderate hypocholesterolemia occurs in cases of steatorrhea, its relation, in this patient, to adult celiac disease, to adrenal cortical hypofunction, or to a possible genetic biochemical defect of lipid metabolism remains conjectural. Withal, the value of this case report is to establish that a man has existed for at least 20 years with a serum cholesterol concentration of 25 to 45 mg. per cent.



# NUTRITION REVIEWS

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## STATUS OF SURVEYS FOR RADIONUCLIDES IN FOODS

This article is a condensation of a report presented to the Food and Nutrition Board of the National Academy of Sciences. It is intended to present a status summary of programs that are presently underway for the monitoring of foods as well as to indicate possible future approaches. No attempt has been made to consider all programs or programs in detail; rather the aim has been to present a broad picture of activities.

*The Overall Problem:* The primary objective of food surveys is to evaluate any possible effect of environmental contamination on the health and well-being of the population. This implies also the prediction of effects in relation to known or predicted levels of contamination.

Studies of environmental contamination in the past have generally fallen into the following categories: (1) surveys, (2) movement in the food chain, and (3) effects on the population.

It is to be emphasized that work in any one of these areas must be interrelated with knowledge of the other two. For example, if it were possible to set a threshold level for a given radiocontaminant in food, then the analytical methods could be simplified and a more thorough coverage of the food supply would be possible for the same expenditure of laboratory effort. It is also clear that knowledge is required from specialists in many disciplines, ranging from the soil chemist, agriculturist, and food technologist, to the medical personnel who must ultimately make the decisions as to effects of given levels of radioactivity in the human population.

*Organization Responsibilities:* The main organizations in the United States actively concerned with some aspect of radiocon-

tamination of food are: Atomic Energy Commission, Department of Agriculture, Department of Defense, Fish and Wildlife Service, Food and Drug Administration, Public Health Service, United States Weather Bureau.

In the past, the Atomic Energy Commission has had the dominant role in regard to surveys of the food chain in the United States and is now carrying forward a comprehensive program. It appears, however, that the Public Health Service is to assume increasing responsibilities; this is in our tradition of separation of regulatory powers and production. There is also a trend for individual states, usually through Boards of Health, to engage in this type of effort.

In August 1959 a Presidential directive stated that the Department of Health, Education and Welfare should "intensify its radiological health efforts and have primary responsibility within the executive branch for the collation, analysis, and interpretation of data on environmental radiation levels." By executive order of the President, a Federal Radiation Council has recently been established to centralize responsibility for providing general standards and guidance to executive agencies in developing operating rules and regulations for radiological health protection.

*Sources of Information:* Information sources on a world-wide basis stem mainly from the following agencies of the United Nations: Food and Agriculture Organization, International Atomic Energy Agency, World Health Organization, U. N. Scientific Committee on Effects of Atomic Radiation.

The above organizations publish many specific reports. For example, the Food and Agriculture Organization will publish shortly the results of a meeting held in December

1959 on contamination in the food chain. The World Health Organization has published a similar report on the genetic effects of low levels of radiation, and a joint report of FAO and WHO on radiochemical analytical methods is now available.

The U. N. Scientific Committee acts as a clearing house for information from various nations and is thus an important source. There are two difficulties that presumably will be overcome in time: (a) raw data (as largely received by the U. N.) are of little use since there must be evaluation and interpretation; (b) since reports are submitted through government channels, there is sometimes loss of contact between the scientists doing the work and the users of the material. The U. N. Committee published an excellent summary report a year or so ago (*Report of the United Nations Scientific Committee on the Effects of Atomic Radiation. Supplement No. 17 (A/3838). New York (1958)*), and in another two or three years will probably publish another.

It should also be mentioned that agencies of other governments publish their own reports and these are numerous indeed. Interpretation is difficult because of lack of standardization of expression and methods and lack of critical evaluation in many instances.

The major sources of domestic information are the Atomic Energy Commission (e.g., *Joint Committee on Atomic Energy (U. S. Congress), Hearings on Fallout from Nuclear Weapons Tests, Vols. 1, 2, 3. May, 1959*); the National Academy of Sciences (e.g., *The Biological Effects of Atomic Radiation, Summary Reports. National Academy of Sciences-National Research Council. 1960*); and the Public Health Service (e.g., *Radio-logical Health Data. U. S. Public Health Service P B 161371-1. April, 1960*).

The emphasis in the domestic reports has been upon immediate presentation of raw data and presentation of individual viewpoints. (persons and organizations).

The data are to be had by anyone for the asking. Serious analyses of data are conspicuous by their absence, a reflection of the hesitancy of scientists in the field to devote the time and energy required to come to grips with the problem.

*Sampling Programs:* It may be of interest to trace briefly the development of sampling programs in this country. There appeared no need for radioactivity assays until after the start of testing of nuclear weapons. In 1947 a sampling program was started at the University of California at Los Angeles that dealt with areas relatively close to the centers of testing. Collections were made of foliage, soil, wild animals, etc., in order to get some idea of the extent of spread of radioactive contamination. In 1948 research laboratories of the Navy began to sample air. In the early 1950's there was recognition of the worldwide spread of radioactive materials with the realization that strontium 90 represented potentially the greatest general hazard. In the period 1951 to 1955 many individual sampling programs were started: each group seemed to have a major interest, i.e., human bones, thyroids, milk, or soil. Most of these programs were supported by the Atomic Energy Commission and, although there was an informal liaison, there was no overall coordination, perhaps because of the feeling shared by most scientists that the problem was not or would not likely become a major one.

In 1956 a high altitude program was started because of the importance of estimating the stratospheric reservoir for prediction purposes. Shortly thereafter, there was considerable development along the lines of whole-body counting for radiocesium in man, and in large samples of feeds and food-stuffs. In 1957 the Public Health Service and the Food and Drug Administration began their programs of food analyses. The limiting factor is the skill, labor, and expense required for reliable analyses and the problem of selection of useful samples.

It may be instructive to describe briefly the procedures for sampling of milk in the United Kingdom since these represent the most intensive effort of its type. Milk is being analyzed for radionuclides of strontium, iodine, cesium, and for stable calcium and stable potassium. The country is divided into ten zones covering 200 milk depots. Special areas where one might expect higher values than normal are also being surveyed; for example, areas of high rainfall or low soil calcium. Actually 40 per cent of the milk supply of the country is being sampled and proportional bulking is used. Milk samples are taken every two weeks.

This procedure is based on the knowledge that the strontium appearing in the milk of a cow represents primarily that which the animal has eaten in the last two weeks. Thus the two-week sampling period can be used with little chance of missing any high points. This is a good example of how knowledge of metabolism can be most useful in the design of surveys.

In the United States there has been no comparable systematic sampling. In 1954 the first series was started using powdered milk from Perry, New York, and liquid milk purchased in New York City; this series was later extended to include four stations. Between 1954 and 1956 there are reports of various types of analyses representing up to 70 stations. The sampling varies from purchase of milk from the grocery store to the use of dried milk obtained at the plant. In 1957 the U. S. Public Health Service started reporting from 12 stations, and it is understood that this network is to be increased to 60. Individual states are beginning to analyze their own samples (New York and Minnesota as examples), and even private organizations (Consumer's Union) have become interested in milk analysis.

Of our food, milk has probably been the most important single item for analysis. This is because milk is produced regularly

all-year-round, is convenient to handle, can be obtained so as to represent small or large areas, and does contain the most important radiocontaminants. *It must be emphasized, however, that the important parameter is the level of contamination of the total diet.* The use of milk as an indicator food does not necessarily imply that milk is the major contributor. As a matter of fact, if nuclear testing is not resumed, milk products may contribute less than 40 per cent of the radiostrontium intake while furnishing up to 80 per cent of the calcium intake. Because the dairy cow utilizes calcium in preference to strontium, it is expected in the future that the calcium of milk will be the least contaminated of all food sources of calcium.

In any sampling program for evaluation of existing environmental contamination, I would rate the following in decreasing order of importance: (1) levels in the human population, (2) levels in the total diet, (3) contribution of individual constituents of the diet, and (4) contribution of precursors of dietary constituents (air, soil, water).

For complete understanding of the system and for capabilities of prediction, items (2), (3), and (4) become as important as item (1).

A word now about the substances that should be considered in evaluation of food-chain contamination. The main stable elements of interest are calcium and potassium; such data are needed because we do not yet know whether strontium 90-calcium relationships or absolute strontium 90 levels are more useful for the purpose, and we do not understand fully the extent to which strontium behavior is governed by calcium. Similar considerations need to be taken into account in regard to cesium-potassium relationships.

The important radionuclides, of course, are those of strontium and cesium, with iodine of interest in short-term situations only, because of its short half-life. Induced activities must be considered, but so far seem to be important only as far as aquatic

food chains are concerned. Carbon 14 must be kept in mind because of possible long-term genetic effects. Fissionable materials as yet appear of secondary importance. Natural radioactive materials are of interest because of the information they can give on steady-state relationships between radioactivity in various parts of the food chain.

*An Approach to the Survey Problem:* As already indicated, monitoring the food supply of a nation for radiocontamination is a complex undertaking. By way of summary, there follows an outline of many of the important parameters that must be taken into account in the logical development of a survey program.

(1) *Nature of Contamination:* (a) Source (weapons, industrial or research usage); (b) Chemical form of nuclide; (c) Physical distribution (worldwide, local, stratospheric, climatic effects); (d) Time factors (single event, continuous production, relationship to growing season).

(2) *Effect of Contamination:* (a) Relationship of levels to presently accepted standards; (b) Possibility of high-risk population groups (aged, infirm, infants, fetuses, occupationally exposed); (c) Primary parameter of hazard (body concentration, local concentration, summation of exposures, genetic effects, somatic effects); (d) Threshold concepts (governs sensitivity of routine measurements needed).

(3) *Basic Sampling Decisions:* (a) Coverage required (geographical, mean levels, extreme levels); (b) Nuclides to be determined (stable and radioactive); (c) Foods to be sampled (reliability of using indicator foods), regional differences and representation, persistence of contamination, weighting for population density.

(4) *Techniques of Sampling:* (a) Ease of handling sampling; (b) Uniformity of product; (c) Uniformity of production (e.g., milk compared to wheat); (d) Point in production-distribution-consumption chain to be sampled; (e) Size of sample, randomness,

scope, frequency; (f) Estimate of variability; (g) Corollary data needed for interpretations (meteorological, dietary, stable nuclides, management practices).

(5) *Techniques of Assay:* (a) Type, reliability, standardization between laboratories; (b) Indirect measurements (e.g., use of cesium 137 to indicate strontium-90 levels).

(6) *Reporting of Data:* (a) Description of sampling; (b) Description of assay; (c) Errors; (d) Standard units; (e) Interpretation.

*Summary Statement:* Until the present time surveys of the food chain in the United States for radioactive contamination have been carried out on a relatively small scale by individual laboratories and investigators. While no claim can be made that representative values are available for the population of the United States, there is enough evidence to demonstrate with reasonable probability that nowhere have levels reached presently acceptable limits.

In the course of events it may well be that closer attention should be given to the monitoring of foods (this situation could arise with or without the likelihood of actual harm from future weapons use, catastrophe, peacetime use of atomic energy, public concern over the wholesomeness of food supplies, or moral issues). It would be hoped that, if the need arises, recognized scientists in areas of nutrition and food technology would join with those of other disciplines to put the work on an increasingly sound basis. In the meantime it behooves organizations and individuals to ensure, to the degree possible, that various committees at all levels of government dealing with matters that involve the food chain, have technical representation by appropriate scientists.

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## NUTRITION OF MAN IN SPACE

*Regimens for feeding astronauts vary primarily with the duration of the expeditions into space. Closed cycle biological systems are required only in space travels of many months' duration.*

Two years after Sputnik I, America's seven hand-picked astronauts completed a training course in how to eat under conditions of weightlessness similar to those found in space (*J. Am. Med. Assn.* **172**, 575 (1960)). While a passenger in a plane flying through a maneuver known as a parabolic curve, each of the astronauts was exposed to zero gravity on several occasions. Thus he became acquainted with handling, among other things, the highly concentrated liquid and semiliquid foods especially designed for him and contained in special squeeze-type bottles and tubes.

In the present state of development of manned space flight, the state of zero gravity has concerned physicians and bio-engineers more than any other problem bearing on nutrition. However, even though man has yet to orbit once around the earth, more emphasis is gradually being placed on the infinitely greater nutritional problems of space travel lasting many hours, days, weeks or even years.

C. T. Randt (*J. Am. Med. Assn.* **172**, 663 (1960)), in describing the impact of space exploration on biology and medicine, mentions the need for development of regenerative systems which use solar energy to provide fluid, food and gaseous requirements to support life during space travels of long duration. He foresees the need of extensive coordinated research into the nutritional requirements of a variety of organisms including man, and into the chemistry of the photosynthetic processes of closed ecologic systems. He even mentions the possibility of studying controlled hypothermia as a means of reducing metabolic requirements for food and fluid and protecting astronauts from stress.

In discussing the physiologic aspects of

hypergravic and hypogravic states, J. E. Ward (*J. Am. Med. Assn.* **172**, 665 (1960)) comments on the problems of nausea and vomiting during weightlessness. He points to experimental observations (Ward, W. R. Hawkins and H. D. Stallings, *J. Aviation Med.* **30**, 151 (1959)) of the deglutition mechanism indicating that, with normal mastication of solids, swallowing of the semisolid bolus of food presents no problem. Poorly chewed solids, however, present difficulty in swallowing and may float above the palate creating a real danger of aspiration (*Nutrition Reviews* **17**, 129 (1959)). Incompetence of the cardiac sphincter of the stomach may be associated with regurgitation of stomach contents when pressure is imparted to the abdomen, especially if large volumes of fluid have been ingested.

Since the rate of passage of materials of varying densities through the intestinal tract is an inverse function of density (F. Hoelzel, *Am. J. Physiol.* **92**, 466 (1930)), food-passage time may be slightly reduced. However, in consideration of the length of the intestine and the surface tension of the semiliquid intestinal bolus (*i.e.*, the opportunity for absorption), Ward, Hawkins and Stallings feel that absorption of metabolites offers no problem.

The medical aspects of ambient radiations of extraterrestrial space, which run the gamut of the electromagnetic spectrum from radio waves through cosmic rays, have been discussed by P. A. Campbell (*J. Am. Med. Assn.* **172**, 668 (1960)). Although not specifically relating these radiations to the nutrition problems of space exploration, he cites both the hazards of these rays and also the usefulness of solar energy in such projects as producing oxygen and water from the soil of the moon, reconstituting

urine into usable water, and even incinerating feces into usable material. Visible light, essential for photosynthesis, provides the energy for biosynthetic gas exchange systems, which, because of weight restrictions, will probably be limited to space stations and lunar colonies. Since direct radiation of astronauts must be avoided, the use of shielding may produce problems of a logistical nature bearing on nutrition.

A comprehensive summary of the many bio-engineering problems of early manned space flight has been presented by R. W. Lawton, B. J. Smith and D. R. Ekberg (*Ann. N. Y. Acad. Sci.* **84**, 31 (1960)). While eliminating long term residence in space and the hazards of radiation from consideration, they mention in detail such matters as launch acceleration, re-entry deceleration, re-entry at escape velocity, landing impact on the earth, weightlessness, noise, vibration, control of the gaseous environment, the relative virtues of four-gas (oxygen, carbon dioxide, water and nitrogen) and three-gas (oxygen, carbon dioxide and water) systems, explosive and rapid decompression, temperature control, instrumentation, communications systems, biological monitoring and safety and reliability standards.

With respect to nutrition, they point out only the necessity of feeding a low-residue diet for several days preceding as well as during a space flight, and suggest that vitamin and mineral supplements for short flights may be neglected. Although nutrition per se is mentioned only briefly, it is impossible to read this enumeration of the physiologic stresses involved in early-manned space flight without recognizing that these pose problems which distinctly bear upon nutrition, especially when projected ahead to more prolonged missions in space.

In an article more directly devoted to nutritional research for the space traveler, B. Finkelstein (*J. Am. Dietet. Assn.* **36**, 313 (1960)) has discussed how, what and when the astronaut will eat (see *Nutrition Reviews* **18**, 100 (1960)). According to the

author, the comparatively simple answer to "how" lies in serving liquids and semisolid foods from collapsible squeeze tubes. A pontube fitted to the end of each squeeze tube will be placed directly in the mouth and food transmitted by applying pressure to the tube. Solids in bite-size form will be removed by hand from a covered container and placed directly into the mouth. For reasons of cleanliness and sanitation, a disposable, lightweight, greaseproof, water-proof polyethylene mitten will be worn over the pressure gloves while eating. According to the author, this is necessary in order to minimize transfer of foreign particles to extremely delicate instruments.

"What" will the astronaut eat is a question the author finds less easily answered, largely because of the psychological role that food plays in a situation of stress. Sensory deprivation tests, achieved by placing subjects in totally dark (comparable to dustless space) soundproof chambers, were conducted with 50 persons for periods ranging from six to 168 hours. Evidence from these tests suggests that food in a situation of stress may be used as a tool to obtain personal satisfaction. Although caloric intakes remained within limits normal for sedentary individuals in a comfortable environment, eating patterns varied from the usual three-meal-a-day cycle to frequent snacking throughout the 24 hours.

Palatability and acceptability were often contrary to usual tastes. Canned orange juice, usually low in acceptability ratings, rated moderate-to-high in isolation. Discrimination among foods in the same food group was impaired; all meats tasted alike and different varieties of canned fruit could not be distinguished. Breads from different flours were frequently considered similar in taste. Thus, removal of visual cues ordinarily associated with eating interferes with the taste and acceptability of food.

Manned balloon flights to altitudes of 100,000 feet have provided a short-term test situation somewhat comparable to



manned space flight. The feeding program for these balloon trials was formulated with certain criteria in mind, among them: (1) the maintenance of high efficiency on the part of the balloonist despite his extremely high activity level and the considerable stress; (2) reduction of food weight to the minimum compatible with good acceptability and adequate nutritional value; and (3) attention to weight and space limitations which restrict food storage and preclude the use of food-service equipment. In discussing the balloon trials held to date, the author seems to feel that food has assumed a significant emotional and social role. She cites the provision of food designed to alleviate rather than contribute to stress as an objective of the space-flight research program.

In the present report, she mentions several types of dietary regimens for the astronaut. The first, a pair of liquid diets each providing approximately 2500 calories and 100 g. protein, contains such items as fruit juices, coffee and chocolate flavoring, high-protein supplements, pureed vegetables, strained meats and a concentrated ice cream mix. Preference ratings on individual items revealed the common pattern of acceptability, *i.e.*, familiar items rated high and unfamiliar laboratory concoctions low, with the latter gradually increasing in acceptability as the tests continued. The second dietary regimen (for the balloonist) contains similar ingredients but includes semiliquid chicken and beef in tubes as well as nuts and pound cake (the last two for psychological reasons). The third regimen, proposed to the National Aeronautics and Space Agency for subjects participating in Project Mercury, includes plans for preflight and in-flight feeding. The preflight diet, designed to alleviate the necessity for defecation during flight, is a high-protein, low-residue diet to be fed for 72 hours prior to take-off. For the meal immediately before take-off, a high-carbohydrate, moderate-protein, low-fat meal conducive to increased altitude

tolerance is proposed. During flight, a variety of sandwiches, meats, fruit sauces and juices, chocolate drinks, cookies and candy is recommended. These foods provide 2000 calories daily with 49 per cent derived from carbohydrate, 16 per cent from protein and 35 per cent from fat. Three thousand ml. of water and a multivitamin preparation (to alleviate stress, according to the author) complete the regimen.

In answer to the final question, "when" will the astronaut be fed, the author recommends eating at two- to three-hour intervals in order to insure optimal nutrition at all times. She points out that *ad libitum* regulation of dietary intake according to the astronaut's desire for food and fluid might mean that an emergency could arise when the subject's state of hydration and nutrition were inadequate. Hence, she stresses the need for frequent scheduled consumption of food during unusually demanding missions.

Two preliminary evaluations dealing in part with specific approaches to the problem of nutrition in space explorations have recently been reported. R. B. Couch *et al.* (*Fed. Proc.* **19**, 13 (1960)) have described successful clinical trials of water-soluble, chemically-defined diets in patients with slowly progressing neoplasms. They found these satisfactory for maintenance of nitrogen balance and for repletion following depletion on a nitrogen-free diet, and superior to isonitrogenous conventional diets if 50 calories per kg. body weight per day were provided. These diets are designed from chemically defined ingredients and are available in any desired physical state applicable to short-term manned space flight.

M. E. McDowell *et al.* (*Fed. Proc.* **19**, 319 (1960)) have described feeding studies in humans designed to test the digestibility and toxicity of algae (*Chlorella ellipsoidea*). For a period of 33 days they fed four men increasing amounts of algae (10, 20, 50, 100, 200 and 500 g. per day) in a diet constant in calories. Their results were not unlike

those previously reported by Japanese workers (*Nutrition Reviews* 17, 238 (1959)). Poor acceptability, even when the algae were mixed with other food, was observed in all subjects at amounts over 100 g. daily. Nausea, fullness, abdominal aching or cramps, mild malaise and headache developed in all subjects. No diarrhea occurred, but stools became bulky, green, dry and hard. No evidences of toxicity were observed, however. These results suggest that *Chlorella ellipsoidea* may not be the organism of choice for prolonged space travel.

Concentrated synthetic diets containing 8 calories per g. are now under development (S. Miller *et al.*, *Wright Air Development Division Technical Report 60-1* (1960)). A high calorie concentration, in addition to the adequate protein, vitamin and mineral composition of most proposed regimens, is achieved through the use of odd-carbon fatty acids. Ketosis is avoided, since from one to as many as three "three-carbon fragments" are produced per molecule. These enter pathways of carbohydrate metabolism and hence do not contribute to ketosis, thereby permitting 60 to 80 per cent of the dietary calories to be derived from fatty acids.

These recent contributions have increased by only a small measure our knowledge of space nutrition since the publication of a symposium on the subject in 1958 (*see Food Technology* 12, 429-459 (1958)). Many of these recent publications, as well as those in the 1958 symposium, dwell on the psychological aspects of food. While this topic may have significance in certain transportation situations, in space flight it deserves less attention than it is receiving. Astronauts

are a highly selected group who can be picked for or trained to acquire an ability to tolerate any dietary regimen, provided it is nutritionally adequate and compatible with the physiology of digestion and the metabolism of the recipient. The experience with long-term feeding of liquid diets (F. Olson *et al.*, *Am. J. Clin. Nutrition* 1, 134 (1953); E. H. Ahrens, Jr., *et al.*, *Ibid.* 2, 336 (1954); D. M. Watkin and J. L. Steinfield, *Fed. Proc.* 16, 343 (1957)) has demonstrated man's ability to adapt to an unusual diet. If the diminutive cosmos of the space vehicle demands, this adaptive ability can be exploited.

Concern, therefore, over the psychological effects of food in near-term space exploration seems unwarranted. Even in the distant future, when more prolonged space travel may be undertaken, the exploitation of man's adaptability would seem more desirable than excessive concern over his emotions as they relate to food. However, the developments in the propulsion field are so rapid that proposals are already being made (K. Ehricke, *Semiannual Meeting, Am. Soc. Mechanical Engineers, Dallas* (1960)) for a manned, nuclear-powered space vehicle, complete with bedrooms, bath, galleys, gymnasium, library and sick-bay, which will carry eight astronauts on a one and one-half year reconnaissance of Mars and/or Venus by 1970-71.

If the engineers can overcome the technical and economic problems involved in propelling a unit for food and food servicing equipment into space, they may also succeed in creating an environment in which consideration of the emotional effects of food will appear more essential than at present.

### IMPORTANCE OF CHOLESTEROL IN THE HUMAN DIET

*College students fed a formula diet free of fat for one week were then given fat and varying increments of cholesterol. Their serum cholesterol concentration rose rapidly until a daily intake of about 600 mg. was reached.*

Now that an increased level of cholesterol in the blood is recognized as one of the

factors having a positive relationship to vascular aging, more attention has been

directed toward factors regulating the concentration of cholesterol in the blood and tissues. Statistical correlation between coronary disease and the quantity of animal fats and protein has been reviewed (*Nutrition Reviews* **18**, 9 (1960)). Not only does the quantity of saturated fat appear to be important, but also the ratio of the amount of this type of fat to the amount of essential fatty acids seems to play a role in cholesterol metabolism (*Ibid.* **18**, 56 (1960)). Other nutrients may also be involved, such as the type of carbohydrate, and certainly the total caloric intake is important.

The actual quantity of cholesterol in the diet is generally considered to be of minor importance. However, a few studies have indicated to the contrary (J. M. R. Beveridge, W. F. Connell, H. L. Haust and G. A. Mayer, *Canad. J. Biochem. Physiol.* **37**, 575 (1959); W. E. Connor and R. E. Hodges, *J. Lab. Clin. Med.* **54**, 800 (1959)).

Beveridge and his group carried their studies a step further in a recent investigation (*J. Nutrition* **71**, 61 (1960)) employing 93 university students, of whom 75 were men and 18 were women.

For eight days these subjects were fed a formula free of fat. After this, the subjects were divided into eight groups on the basis of their serum cholesterol concentration, and were given a modified formula for another eight days containing a cholesterol-deficient butter oil (which made up 30 per cent of the calories at the expense of carbohydrate) and adequate amounts of essential vitamins. To each of the eight diets was added an increment of crystalline cholesterol ranging from zero to 1600 mg. per 950 calorie portion. This was homogenized into the formula and the subjects were given a sufficient amount of it to maintain their weight at a constant level. Total serum cholesterol was determined on days zero, four, eight 12 and 16.

During the initial period when fat was omitted from the diet, the concentration of serum cholesterol declined rapidly, the averages being: initial, 201 mg. per cent;

day four, 147 mg. per cent; and day eight, 146 mg. per cent. The addition of fat and cholesterol was followed by a marked increase in the concentration of cholesterol in the blood. The authors estimated the average daily intake of cholesterol to be 13 mg. in the lowest group and almost 4500 mg. in the highest group.

Although the response of serum cholesterol concentration was rapid as the daily cholesterol intake increased up to 600 mg., there was a statistically significant rise in serum cholesterol only up to an intake of approximately 2500 mg. Greater amounts of cholesterol in the diet did not increase the cholesteroemia.

The authors concluded that the concentration of serum cholesterol in man is affected by dietary levels of this sterol, but that some moderately effective control mechanism exists in healthy young people. Presumably this mechanism is impaired in hypercholesterolemic families, in diabetics, and in some other conditions. This would explain the failure of numerous investigators to find a hypercholesterolemic effect of additional cholesterol added to the usual diet of healthy volunteers. In the present study, there was considerable leveling off of the response when more than 600 mg. of cholesterol was fed. This amount approximates the cholesterol content of the usual diet of many persons.

The demonstration of a hypercholesterolemic effect of dietary cholesterol is important to any study of diets and diseases.

This study was well planned, but had several minor defects. The short duration of observation seems undesirable, but may have been necessary. In addition, it might have been more convincing had the initial diet been one containing 30 per cent of fat devoid of cholesterol; then the addition of cholesterol alone would have been the sole variable. Also, one always wonders whether results obtained on a formula diet apply to a diet of varied foods. Nevertheless, the results are of considerable interest.

## COPPER IN INFANT NUTRITION

*A controlled study of prematurely born infants fed a low-copper diet failed to yield evidence of copper deficiency. Processed baby foods provide adequate amounts of copper.*

In recent years, a number of clinical reports have appeared in the literature describing a syndrome characterized by microcytic hypochromic anemia, hypoproteinemia and hypocupremia (*Nutrition Reviews* 15, 72 (1957)). Although this syndrome is rare, its occurrence in young infants has produced a revival of interest in the role of copper in infant nutrition.

A deliberate attempt has been made to induce copper deficiency in young infants by J. F. Wilson and M. E. Lahey (*Pediatrics* 25, 40 (1960)). Twelve prematurely born infants (average birth weight 1200 g.) were selected. Seven were given a milk formula containing 0.07 mg. of copper per liter. This formula was fed for an average of nine weeks, the only supplement being a commercial multivitamin preparation. Records of intake were kept, and it was estimated that the daily intake of copper was about 0.015 mg. per kg. body weight per day. This level of intake is far below that usually recommended (*National Academy of Sciences-National Research Council Publication 589. Washington D. C. (1958)*), and is commensurate with that known to produce copper deficiency in swine and rats.

Four of the remaining five infants were given a milk formula containing five to eight times as much copper, while the fifth was given a supplement of copper sulfate sufficient to raise the daily intake to 45 times that of the low-copper group.

Upon dismissal from the hospital, all of the infants were given a daily supplement of iron, and foods other than milk were allowed. The observation period was continued until age six months. However, at this age biochemical data are reported for only seven of the original 12 infants.

All of the infants were carefully observed with respect to growth performance. Determinations of blood hemoglobin content, serum iron and copper, and serum proteins were made at intervals. At the end of the study the low-copper group was compared to the high-copper group.

No difference in growth performance between the two groups could be detected. With one exception, the general condition of the babies fed a low-copper diet was good. This infant developed megaloblastic anemia at age 17 weeks; serum iron and copper levels were normal, and there was a prompt response to folacin therapy. In the author's opinion, this circumstance was not due to the low-copper diet.

Both groups reached a low level of hemoglobin at about eight weeks of age, with a gradual upward trend after the fifteenth week. By six months of age the high-copper group had a higher blood hemoglobin content (11 g. per cent) than did the low-copper group (9 g. per cent), although there was no appreciable difference in serum iron content.

Serum copper levels remained low (0.04 to 0.09 mg. per cent) until about ten weeks of age; by six months of age these had risen to the range of 0.1 to 0.2 mg. per cent. Infants receiving the low-copper formula had, on the average, a slightly higher serum copper level than did the others. In both groups, there was a good correlation between serum copper and alpha-2-globulin levels. Hypoalbuminemia did not occur.

When all of the observational data are considered, it is apparent that varying the dietary intake of copper was without effect during the first two months of life. The babies fed the low-copper diet appeared normal in every respect. By six months of age, however, this group had a somewhat

lower hemoglobin level, though hypocupremia was not present, nor were other manifestations of copper deficiency.

This reported failure to provoke copper deficiency on a diet known to produce deficiency in animals raises certain interesting points. The first of these is growth rate. The authors deliberately selected prematurely born infants for their study, since growth is rapid in such infants. However, human infants grow much more slowly than do either rats or pigs if growth is portrayed in terms of the percentage or relative growth rate. Indeed, most young mammals grow at a much faster rate than the human. It could be reasonably anticipated, therefore, that the dietary requirements of most nutrients on a daily per kg. basis would be less for the human infant.

Thus the question as to what would have happened had an even lower copper diet been fed or had the present dietary regimen been extended, is still unanswered.

The diet offered to the infants in this study after two months of age is not specified (nor, for that matter, is analysis of the multivitamin preparation available), and no estimate of the copper intake during this period is provided by the authors. In this connection, a recent report by G. Hughes, V. J. Kelly, and R. A. Stewart (*Pediatrics* 25, 477 (1960)) is of importance. These authors assayed some 2600 samples of 86 different processed baby foods for copper content. The foods in question, although prepared by one manufacturer, were processed in various parts of the country.

Baby cereals and beef liver are the richest

source of copper in this group of foods, with average values in the range of 0.3 to 2.6 mg. copper per 100 g. (as taken from the container). Other meat products and egg yolk contained an average of 0.07 to 0.18 mg. per 100 g., mixed vegetable and meat soups 0.06 to 0.17 mg., vegetables 0.08 to 0.16 mg., fruits 0.06 to 0.13 mg., and desserts 0.05 to 0.08 mg. per 100 g. There were no significant differences ascribable to locality of packing or to the type of container (tin, glass) used.

The authors conclude that the copper intake for infants consuming an average amount and variety of processed baby foods would be at least 0.05 mg. copper per kg. body weight per day. They also point out that a diet consisting of milk of low-copper content supplemented only by foods having the lower copper content would provide only two-thirds of this amount.

While Wilson and Lahey's study does not establish the minimum requirement of copper for young infants, it does document the fact that they can thrive, for short periods at least, on intakes well below those currently recommended. How long they would have continued to thrive on such a diet is a most important question, for the majority of infants reported in the clinical literature as suffering from hypocupremia have subsisted largely on milk for periods of six to 12 months or longer. The analyses reported by Hughes, Kelly, and Stewart indicate that Wilson and Lahey's subjects conceivably could have received adequate amounts of copper from supplemental baby foods in the latter period of the study.

## CARDIAC DISEASES IN THREE RACIAL GROUPS IN SOUTH AFRICA

*In Capetown, the Bantus have less coronary disease but more rheumatic heart disease and pericarditis. European residents have a reversed incidence. Dietary factors may contribute to these findings.*

Although much has been written about the incidence of coronary vascular diseases

in a number of different countries, the relative roles of hereditary environment, diet,

occupation, and emotional stress are difficult to assess. In the United States, H. I. Russek (*J. Am. Med. Assn.* **171**, 503 (1959)) found that the emotional response to insecurity, frustrations and restlessness seems to be a more important factor than diet, the use of tobacco, heredity or obesity. In Japan and Thailand, O. J. Pollak (*Am. J. Clin. Nutrition* **7**, 502 (1959)) found the incidence of coronary disease to be the inverse of the amount of animal fats eaten by the populace. Despite these exceptions, most students of nutrition agree that dietary fats of a saturated nature if not balanced by an adequate intake of certain unsaturated fatty acids (linoleic acid equivalent) may contribute to hypercholesterolemia and to vascular disease.

In Capetown, South Africa, three groups of people differing in ethnic origin, social-economic status, and dietary customs live in close proximity. The Bantus are a tribe of middle African origin who live in relative poverty and eat a diet containing few animal fats and modest quantities of protein. The "Cape Coloreds" are a mixture of Asiatic, European and African peoples who occupy an intermediate position in the social economic structure and eat a diet containing more fats and proteins. The white population is of European origin. Their diet contains a great deal more of these expensive foods.

Quite naturally this situation has attracted students of heart disease to conduct surveys of several types (B. Bronte-Stewart, A. Keys, and J. F. Brock, *Lancet* **II**, 1103 (1955); Bronte-Stewart, *Brit. Med. Bull.* **14**, 243 (1958)). Although these studies initially suggested that the Bantus had a remarkably low incidence of coronary vascular disease, closer scrutiny of the data discloses that these people have types of heart disease other than atherosclerotic.

In a similar study of the three racial groups in Capetown, V. Schrire has compared the Bantus, the "Cape Coloreds",

and the whites (*Am. Heart J.* **59**, 835 (1960)). The author chose data from hospital records of over 1300 patients of all ages, both sexes, and all three racial groups. These patients had been hospitalized in the teaching beds of two hospitals (898 beds) over a period of five consecutive years. They had had electrocardiographic tracings because of suspected heart disease and, in nearly all instances, the tracings were examined by the author. Fifty-seven per cent of the records were from whites, 37 per cent from "Cape Coloreds", and 6 per cent from Bantus.

The diagnoses were tabulated according to standard classifications and by racial origin. As one might anticipate, the white people had a much higher incidence of coronary vascular disease (26 per cent) than did the "Cape Coloreds" (13 per cent) or the Bantus (1 per cent). Interestingly enough, the incidence of hypertension was similar for all three groups (46, 51 and 35 per cent, respectively.) However, rheumatic heart disease occurred more commonly in the "Cape Coloreds" (25 per cent) and in the Bantus (20 per cent) than in the whites (12 per cent).

Beri-beri and "cardiopathy presumably of dietary origin" occurred in 0.5 per cent of the white people, in 1.5 per cent of the "Cape Coloreds" and in five per cent of the Bantus. Most of the patients of all three races with this diagnosis were considered to be alcoholic. Another strange finding was an incidence of 15 per cent of pericarditis in the Bantus compared with only 2 per cent in the "Cape Coloreds" and 0.5 per cent in the whites.

These findings would suggest that the white population, by virtue of their diet or their heredity or their social-economic status, had relative protection against rheumatic fever, nutritional heart disease and pericarditis, but had an increased susceptibility to coronary vascular disease. Conversely, the Bantus, presumably for



the same reasons, were more susceptible to rheumatic fever, pericarditis, and nutritional heart disease, but were relatively immune to atherosclerosis of the coronary vessels. An intermediate position was held by the "Cape Coloreds", except for a modest increase in the incidence of hypertensive disease.

A. R. P. Walker and H. Grusin (*Am. J. Clin. Nutrition* **7**, 264 (1959)) compared the incidence of coronary disease to that of cerebral vascular disease in the Bantus of Johannesburg, South Africa. They concluded that, although accurate statistics were difficult to obtain, there seemed to be a higher incidence of cerebral than of coronary vascular disease in these people. They suggested that studies of salt intake in the diet and of the incidence of essential hypertension might be revealing.

The present report by Schrire deals in part with hypertension. It was found that its occurrence in the Bantus was slightly less than in the other groups. Furthermore, its reasonably even distribution among all

three racial groups was comparable to the 21 per cent of all hospitalized patients in India and 26 per cent of all hospitalized patients in the United States. Thus these data suggest that neither diet nor social-economic status nor racial origin plays a critical role in the etiology of essential hypertension.

This study, although derived from crude data, provides information pertinent to the interpretation of other reports. It suggests that as the social-economic status increases and the diet becomes more abundantly supplied with foods of animal origin, the incidence of pericarditis and rheumatic fever declines in a reciprocal relation to that of coronary atherosclerosis. One might compare these disorders to opposite ends of a teeter-totter in which hypertensive disease serves as a fulcrum. These speculations may aid in formulating the designs of additional studies which will aid in the selection of diets compatible with a low incidence of all types of heart disease.

### NEW ANTIMETABOLITES OF VITAMIN B<sub>12</sub>

*In a search for antimetabolites of vitamin B<sub>12</sub>, 15 promising compounds were found. These included benzimidazoles, diazobenzimidazoles, purines, pyrimidines, pteridines and niacin derivatives.*

Perhaps one of the most challenging forms of medical investigation involves the concept of antimetabolites. In recent years we have observed the dramatic effects of a few, such as the sulfonamides, the coumarin drugs, the anti-folic acids and the antibiotics. D. W. Woolley has done much to turn the imagination of investigators toward studies of new antimetabolites (*A Study of Antimetabolites. John Wiley & Sons, Inc., New York (1952)*). He discussed a compound, 1,2-dimethyl-4,5-diaminobenzene, which he considered to be a precursor of two important vitamins, riboflavin and vitamin

B<sub>12</sub>. The latter has been studied intensively because of its unique role in the processes of hematopoiesis and in the metabolism of the central nervous system. Evidence suggests that it also is vital to the cellular functions of the kidney, the muscles and other tissues. Although dimethyl-diaminobenzene was shown to have slight anti-vitamin B<sub>12</sub> activity, there has been no antimetabolite which could effectively inhibit all of the actions of this vitamin.

Recently M. Bodian (*Pediat. Clin. N. Am.* **6**, 449 (1959)) reported that large doses of vitamin B<sub>12</sub> caused regression of neuro-

blastomas. Another report by Woolley linked neoplastic disease with vitamin B<sub>12</sub> (*Proc. Nat. Acad. Sci.* **39**, 6 (1953)). He found that spontaneous mammary tumors of mice seemed to be synthesizing vitamin B<sub>12</sub>. L. Smith (*Nature* **181**, 307 (1958)) studied some analogues of vitamin B<sub>12</sub>, and another group employed 2-ethyl-2,3-naphthimidazole-4,9-dione to suppress the growth of mammary carcinoma of mice.

These considerations led to a study by G. M. Timmis and S. S. Epstein (*Nature* **184**, 1383 (1959)), in which they employed approximately 100 analogues of vitamin B<sub>12</sub>. The test organism was *Euglena gracilis*, a flagellated green alga whose growth can be measured readily (S. H. Hutner *et al*, *Proc. Soc. Exp. Biol. Med.* **70**, 118 (1949)). Those chemical compounds, which in concentration of 500  $\mu$ g per ml. induced 50 per cent inhibition of this organism, were selected for further study. The test systems compared growth of *E. gracilis* in the presence of 0, 10, 100 and 1000  $\mu$ g. with varying quantities of the antagonists added. Results were expressed as that quantity of an antagonist needed to induce 50 per cent inhibition of growth at each concentration of vitamin B<sub>12</sub>.

The compounds included six structural types: benzimidazoles, diazobenzimidazoles, purines, pyrimidines, pteridines and niacin derivatives. In each of these groups, inhibition of growth in the presence of 10  $\mu$ g. of vitamin B<sub>12</sub> was accomplished by addition of 50 to 200  $\mu$ g. of the antagonist. This would indicate that these compounds are sufficiently active to justify animal studies.

Of particular interest is the finding that derivatives of pteridines and of niacin have anti-vitamin B<sub>12</sub> activity. Unlike the benzimidazoles and the purines, they are not analogous in structure to any part of the vitamin B<sub>12</sub> molecule.

Although 6-mercaptopurine, used in therapy of acute leukemias, has an inhibitory effect upon the synthesis of nucleic acids and proteins, the authors considered that it may exert some antivitamin B<sub>12</sub> action also since it was a potent inhibitor in the present study.

Development of new and potent inhibitors of vitamin B<sub>12</sub> undoubtedly will prove to be important in the study of neoplastic diseases as well as of normal hematopoiesis. This report should stimulate many investigative efforts.

### EFFECTS OF FORCE FEEDING

*Rats eating ad libitum have less body fat than those force fed the same amount of feed.  
Force feeding produces a threefold increase in activity of enzymes involved in lipogenesis.*

During the past few years a number of investigators have reported that when animals are force fed they show altered physiological effects. A large amount of work in this area has been done by C. Cohn and associates, who found that when rats are tube fed they deposit more fat in their bodies than animals eating ad libitum. This was true even though the force-fed animals received only enough food to produce a rate of gain in body weight equal to that of the ad libitum controls.

Their attention was directed to the above

problem by the observation that adrenalectomized animals deposited much more body fat when force fed than did the control animals. The work with adrenalectomized rats was stopped when it was observed that normal animals behaved in the same way (C. Cohn, D. Joseph and E. Shrago, *Metabolism* **6**, 381 (1957)). Normal rats force fed enough food to produce the same weight gain over a two-week period as animals normally eating had approximately twice as much body fat as the controls.

In order to determine whether the in-

creased body fat in the force-fed animals was caused by their receiving all of their food twice a day, the investigators fed three separate groups of animals by stomach tube in 2, 3 or 4 portions, respectively, during the day. During the two-week trial period the average weight gain for the animals in these groups ranged from 61 to 64 g. The body fat content, however, was the same in all three groups.

In another trial (Cohn *et al.*, *loc. cit.*), rats were trained to eat their daily quota of food within a two-hour period (a semipurified diet suspended in water). However, these animals had the same percentage of body fat as the controls with access at all times to the same diet except in the dry state.

Finally, to determine whether the difference in the consistency of the diet had any effect on the body composition of the ad libitum-fed rats, a group of ten animals was fed the dry ration and another group of ten received the same diet mixed with water. These rats were compared with a group of ten receiving the diet mixed with water but force-fed in amounts which produced the same rate of gain as that of the group receiving the solid diet on an ad libitum basis. The consistency of the diet had no effect on the percentage of body fat in the ad libitum groups. However, the force-fed group had a larger amount of body fat than the average for all ad libitum-fed rats (17 vs. 12 per cent).

Since the tube-fed animals were depositing more fat, it was obvious that they were metabolizing fewer calories than the ad libitum-fed controls. For this reason, Cohn and associates (*Endocrinology* 62, 251 (1958)) examined a number of parameters of thyroid function. All of the animals, as in the previous studies, weighed between 150 to 160 g. at the start of the experiment. The force-fed animals were adapted to the liquid diet over a period of one week in order to avoid "food shock." They were fed twice daily in the early morning and late afternoon. After two weeks of such feeding,

the ad libitum controls had an average body weight of 230 g. while the force-fed rats averaged 227 g. At that time, the thyroid glands of the ad libitum controls took up 7.94 per cent of the administered  $I^{131}$  in 24 hours, whereas the thyroids of the force-fed animals took up only 1.71 per cent.

As a further measure of thyroid activity, the amount of administered  $I^{131}$  bound to the plasma protein (PBI) was determined. The ad libitum-fed rats incorporated one and one-half times as much  $I^{131}$  into PBI as the force-fed rats. The concentration of injected  $I^{131}$  iodide in 1 mg. of thyroid tissue over the concentration in 1 ml. of serum (T/S ratio) was the same for both the force-fed and ad libitum-fed animals. According to the authors, these data suggested that force feeding produces hypothyroidism, which is secondary to a decreased production or release of thyroid-stimulating hormone from the anterior pituitary gland.

In another set of experiments, Cohn and Joseph (*Am. J. Physiol.* 196, 965 (1959)) used the paired-feeding technique; the rats on the ad libitum regimen and those that were force fed received the same amount of diet. The latter were fed by stomach tube twice each day. After two weeks of such feeding, the animals were sacrificed and the tail and hair removed and discarded. Carcass analyses indicated that, although both groups of rats showed the same increase in body weight (58 and 56 g. respectively), the force-fed animals had 26 g. of fat in their bodies, whereas the ad libitum-fed animals had 19 g.

The rapid accumulation of fat by the force-fed rats was sharply demonstrated upon comparison with animals that had been sacrificed prior to the actual start of the experiment. (All animals had been maintained on their respective feeding regimens for one preliminary week.) The force-fed rats sacrificed at that time had an average of only 13 g. of fat in their bodies, and the ad libitum-fed rats had 15 g. The increase in body fat during the actual experiment

was, therefore, three times as great in the force-fed rats as in the controls.

Calculations by the investigators showed that 24 per cent of the tissue laid down by the force-fed animals was fat, whereas fat accounted for only 7 per cent of the tissue acquired by the rats fed ad libitum.

The bodies of the above animals were divided into skin, viscera and residual carcass, and each of these was analyzed separately. The results indicated that the skin of the force-fed rats showed the greatest relative increase in fat (eight times greater than in the ad libitum controls). There was relatively little difference in the rate of accumulation of fat in the viscera, while the fat increase in the residual carcasses of the force-fed animals was twice that of rats fed ad libitum.

In order to determine whether the more rapid presentation of food to the body, as in force feeding, has any effect on metabolic pathways, Cohn and Joseph (*Am. J. Physiol.* **197**, 1347 (1959)) studied the hexose-monophosphate shunt activity in the livers and epididymal fat pads of the force-fed and control animals. The glucose-6-phosphate dehydrogenase activity was two to three times greater in both the liver and adipose tissue of the tube-fed animals than in the tissues of the ad libitum-fed controls. The investigators pointed out that the shunt is an important source of reduced TPN and that fatty acid synthesis is dependent, among other things, upon the generation of the reduced nucleotide. They conclude from this work that forced feeding of meals is associated with adaptive enzyme changes favoring lipogenesis.

The authors suggest that the rate of ingestion of foodstuffs influences the intermediary metabolic pathways to which the food is subjected. As evidence for this, they refer to the work of N. Werthessen (*Am. J. Physiol.* **120**, 458 (1937)) and J. Tepperman, J. R. Brobeck and C. N. H. Long (*Yale J. Biol. Med.* **15**, 855 (1942)), who observed lower respiratory quotients in rats trained

to eat their entire day's ration during a restricted period of time than in the ad libitum-fed controls. Similar differences in the respiratory quotient of liver slices from animals trained to eat their food in a short period of time were observed by V. C. Dickerson, Tepperman and Long (*Yale J. Biol. Med.* **15**, 875 (1942)). The above results were interpreted as indicating excessive deposition of fat in animals adapted to eating their food within a short period. This adaptation probably stems from the development of "alternate and perhaps more 'efficient' pathways."

To explore the difference in rate of food ingestion on the development of a metabolic disease, Cohn, R. Pick and L. N. Katz (*Circulation* **20**, 969 (1959)) permitted a group of chicks access to food for an hour in the morning and for another hour in the afternoon. The other group was permitted the amount of feed consumed by the first group but ate at will. The diet contained 0.5 per cent cholesterol, 5 per cent cottonseed oil and 20 per cent protein. After five weeks, the chicks eating only twice a day had an average serum cholesterol of 584 mg. per cent while that of the other group was 294. The thoracic aortas of the chicks eating twice a day had a higher incidence of atherosclerosis (100 vs. 44 per cent), which was also more severe. Microscopic examination showed that 14.6 per cent of the coronary vessels in "meal eaters" (receiving food once or twice a day) had atherosclerosis while only 2.2 per cent of the vessels in the nibblers were so affected.

In another experiment, half of a group of chicks that had developed elevated levels of blood cholesterol were fed the non-cholesterol ration for one hour each day. These chicks cleared their coronary arteries only one-third as rapidly as chicks eating the non-cholesterol diet throughout the day.

The studies of Cohn and associates are intriguing. It must be pointed out, however, that in their earlier work (*Metabolism* **6**, 381 (1957)) they reported that even when the

day's food quota was given in four equal stomach tubings, the rats showed the same accumulation of body fat as did those receiving all of their food once a day by stomach tube. Cohn and Joseph explain this apparently discordant observation by suggesting that they "did not feed frequently enough nor spread the feedings over a sufficient number of hours daily" (*Ibid.* 9, 492 (1960)). However, of greater consequence was their report (*Ibid.* 6, 381 (1957)) that when rats were trained to eat their day's food in an hour, their body fat content was the same as that of the ad libitum-fed controls. If credence is to be given the suggestion that the rate of ingestion of food influences metabolic pathways, then the above discrepancies should be clarified by experimental observations.

Another criticism of the work of Cohn and associates is that within a two-week experimental period changes may be seen which on further observation disappear. It is entirely possible that here, as in many other situations, a two-week period is too short for establishing an equilibrium condition.

One factor discussed by Cohn and Joseph (*Metabolism* 9, 492 (1960)) is that the forced-fed rat "conserved more calories, which were retained as fat, than his nibbling partner eating ad libitum." Actually, the differences in the caloric balances of the two groups of rats may be relatively small, about 5 Calories per rat per day. The latter was calculated from the composition of the body weight gained during the two-week experimental period (*Am. J. Physiol.* 196, 965 (1959)). If possible, a caloric balance should be carried out to clarify this point.

Finally, it should be pointed out that

when rats had access to a cholesterol-containing diet for three hours each day, they had cholesterol levels of 61 mg. per 100 ml. of serum, while animals with access to the same diet 24 hours a day had cholesterol levels of 85 mg. (R. Okey, G. Scheier and M. Reed, *J. Am. Diet. Assn.* 36, 441 (1960)). The animals used in this later work were started on the experiment at weaning and maintained for seven weeks. The preceding observation makes it all the more imperative to clear up the questions that have been directed at the evidence used in building a hypothesis as to the effect of meal-eating as contrasted to nibbling.

Regardless of the above criticism, these studies merit consideration by both animal experimentalists and those interested in human nutrition since they suggest that by altering the eating pattern of an animal one may be able to alter a variety of physiological functions or speed up the development of an abnormality (atherosclerosis). Thus science may be provided with a new tool comparable to the "thyroid stress" used so frequently in a variety of studies.

Cohn's work also suggests that pair-fed rats and those permitted to eat ad libitum may not be as comparable as investigators in the past have thought them to be and that caution should thus be exercised in interpreting results of paired-feeding experiments, especially those lasting for only a few weeks.

On the basis of the above work, it would appear justifiable to record the eating pattern of human beings when dietary surveys are included in a medical or biochemical study.

### NUTRITIONAL MUSCULAR DYSTROPHY

*The development of muscular dystrophy in calves on a low vitamin E diet was intensified by feeding cod-liver oil. The resulting low serum magnesium levels may have accentuated the muscle changes.*

Although muscular dystrophy develops in many different species when fed diets de-

ficient in alpha-tocopherol, the severity of the lesions and the time required for their

production in the different species varies a great deal. D. C. Maplesden, J. D. Harvey and H. D. Branion (*Canad. Vet. J.* **1**, 10 (1960)) formulated a diet which produced muscular dystrophy in calves, but the time required for the development of the dystrophic lesions was extensive. Calves have been shown by other investigators to be quite resistant to muscular dystrophy when compared to other species such as guinea pigs, sheep and goats (G. K. Davis and L. A. Maynard, *J. Dairy Sci.* **21**, 143 (1938)). Thus an extended feeding period may be required to produce muscular dystrophy in calves on a diet low in unsaturated fatty acids and the tocopherols.

However, K. L. Blaxter, W. A. Wood and A. M. MacDonald (*Brit. J. Nutrition* **7**, 34 (1953)) demonstrated that cod-liver oil added to the diet of the calf would nullify the action of DL-alpha-tocopherol in the diet, and this effect of the unsaturated fatty acids in cod-liver oil apparently extends to the tocopherols already present in the animal body.

The report by K. Schwarz and C. M. Foltz (*J. Am. Chem. Soc.* **78**, 3292 (1957); see also *Nutrition Reviews* **18**, 193 (1960)) that selenium was an integral part of Factor 3 and that inorganic selenium salts were remarkably effective in protecting against dietary necrotic liver degeneration in rats has rapidly led to studies with other species. Selenium was reported by O. H. Muth *et al.* (*Science* **128**, 1090 (1958)) to prevent muscular dystrophy in lambs just as effectively as vitamin E, but the changes in tissue mineral element content with muscular dystrophy reported by many investigators suggest the condition is not a simple vitamin E deficiency.

In an effort to differentiate the action of selenium and alpha-tocopherol as nutrients which prevent muscular dystrophy, Maplesden and J. K. Loosli (*J. Dairy Sci.* **43**, 645 (1960)) made use of the diet described by Maplesden, Harvey and Branion (*loc. cit.*), but intensified the production of a tocoph-

erol deficiency by adding cod-liver oil. While no gross evidences of muscular dystrophy appeared (with one possible exception), all of the calves not supplemented with alpha-tocopherol showed low plasma tocopherol levels, averaging 108  $\mu$ g. of total tocopherol per 100 ml. of plasma, as compared to 639  $\mu$ g. found in calves receiving supplementary D-alpha-tocopherol acetate.

All of the calves maintained on the alpha-tocopherol-deficient diet plus cod-liver oil for more than 90 days showed, upon histological examination, Zenker's degeneration of the skeletal muscles and degeneration of the Purkinje fibers of the heart. On the other hand, none of the animals receiving tocopherol had any evidence of muscular dystrophy or heart lesions. Interestingly enough, the addition of 1 p.p.m. of selenium to the diet (which already contained 0.3 p.p.m. of selenium) had no effect upon the development of the muscular dystrophy.

It was quite evident that the addition of cod-liver oil produced much more extensive lesions than observed by Maplesden, Harvey and Branion (*loc. cit.*), but the calves continued to eat and grow normally. The development of muscular dystrophy without gross clinical symptoms makes it readily understandable how sudden exercise in the spring could aggravate or precipitate Zenker's degeneration.

It was interesting that in the experiments of Maplesden and Loosli the blood magnesium levels dropped in those calves receiving cod-liver oil. Since B. A. Dehority *et al.* (*J. Animal Sci.* **17**, 1183 (1958)) had observed that additions of cod-liver oil to the diet of calves caused a decrease in blood serum magnesium and since the basal diet used by Maplesden and Loosli contained 20.2 mg. of magnesium per 100 ml. of liquid diet (an adequate level), it may be suggested that the cod-liver oil was interfering with magnesium utilization, possibly through the formation of magnesium soaps.

Since Blaxter, J. A. F. Rook and Mac-



Donald (*J. Comp. Path. Therap.* **64**, 157 (1954)) did not observe any degeneration of the Purkinje fibers in calves that developed a hypomagnesemic tetany on a magnesium-deficient diet fortified with tocopherol, Maplesden and Loosli suggest that the intensification of the Zenker's degeneration and the changes in the Purkinje fibers may represent a synergistic effect brought on by the low levels of tocopherol and magnesium. Certainly the possibility that the unsaturated fatty acids of cod-liver oil not only

interfere with vitamin E utilization but also with mineral element absorption is an interesting one.

The ineffectiveness of selenium in the experiments of Maplesden and Loosli emphasizes the need for basic information on the mechanism whereby dietary constituents are utilized. The complexity of the factors which interfere with absorption and utilization suggests that this field of research, especially respecting the functions of trace elements, has hardly been scratched.

### FATTY LIVER INDUCTION BY OROTIC ACID

*The orotic acid fatty liver is prevented by a natural diet or by adenine and must be an indirect result of deranged nucleotide metabolism.*

Orotic acid (6-carboxy-2,4-dihydroxypyrimidine) has been shown to be an intermediate in pyrimidine nucleotide synthesis in both microorganisms and higher animals (I. Lieberman and A. Kornberg, *J. Biol. Chem.* **207**, 911 (1954); Lieberman, Kornberg and E. Simms, *Ibid.* **215**, 403 (1955)). It is apparently formed from carbamyl aspartate and converted to orotidine-5-phosphate and to uridine-5-phosphate, thus entering intimately into nucleotide synthesis and metabolism. It is probably formed in small amounts in many tissues and is a minor constituent of many dietary substances such as milk.

It was surprising, therefore, to find that when rats were weaned on purified diets containing 1 per cent or less of orotic acid, they developed fatty livers (S. B. Standerfer and P. Handler, *Proc. Soc. Exp. Biol. Med.* **90**, 270 (1955)). Moreover, the mechanism of fatty liver production was not apparent since the usual lipotropic agents, such as choline, methionine, folacin or cobalamine did not prevent the fat accumulation.

In order to clarify these observations, R. E. Handschumacher and co-workers (*Proc. Nat. Acad. Sci.* **46**, 178 1960)) studied the production of fatty livers by

orotic acid and its relationship to other nutritional and biochemical factors. Weanling rats were given either a commercial dog chow diet or a purified diet containing 18 per cent casein, 72.8 per cent sucrose, 2 per cent corn oil, 2.2 per cent vitamin mixture and 5.0 per cent salts, with additional choline chloride at 0.3 per cent of the total. When 1 per cent orotic acid was included in the purified diet, fatty livers developed maximally within seven days. In all other respects, these rats appeared to be normal except for a somewhat lower growth rate.

Since 1 per cent orotic acid in the dog chow diet did not induce fatty livers, a search was made for factors which might be responsible for a possible protective agent. When it was found that, of many substances tried, only 0.25 per cent of adenine sulfate or compounds containing adenine would prevent or reverse the orotic acid fatty livers, it seemed evident that nucleotide metabolism was involved in some way.

Several experimental results appeared to confirm this notion. The amounts of ribonucleic acid in livers of animals fed either the purified orotic-acid-supplemented diet plus adenine or the dog chow diet were

greater than in those of animals fed the purified diet alone or this diet plus only orotic acid. When the rate of formation of uridylic acid from orotic acid was measured (by evolution of  $C^{14}O_2$  from orotic acid-7- $C^{14}$ ) in liver slices, a lower rate was found for livers of animals fed orotic acid and an increase for those with supplemental adenine or fed the dog chow. Moreover, conversion of adenine to adenine nucleotides was depressed in the  $106,000 \times$  g. supernatant solutions of livers from rats fed 1 per cent orotic acid, but was, however, not restored by adenine or dog chow even though the fat accumulation was prevented. It was also noted that degradation of orotic acid-2- $C^{14}$  was markedly depressed in liver slices of rats with dietary orotic acid, but was then greatly enhanced by removal of the orotic acid or inclusion of adenine.

From these results, the authors suggest that the fatty livers are caused by a reversible metabolic imbalance brought about by excess orotic acid even though this substance is a normal metabolic intermediate.

Prevention of this effect by adenine suggests that the action of orotic acid lies in stimulation of additional synthesis of nucleic acids, thus reducing the pool of nucleotides and possibly bringing about shortages of co-enzymes of certain purines and pyrimidines, which are not replaced in the purified diet. In particular, the adenine nucleotides appear to be limiting in this case, and it is possible that an adenine coenzyme is involved in some stage of the removal of lipid from the liver.

The authors also call attention to a report (L. E. Hallanger, J. W. Laakso and M. O. Schultze, *J. Biol. Chem.* **202**, 83 (1953)) that, although human milk contains insignificant amounts of orotic acid, cows' milk may contain it to the extent of 0.2 per cent of the solids. Although it is doubtful that this amount would lead to any abnormalities in liver function, especially with the additional nutrients of the milk, it is a possibility which may deserve some consideration.

### SOME FUNCTIONS OF VITAMIN E

*Premature infants and all newborns have low serum levels of tocopherol without incurring hemolytic anemia. The biochemical effects of vitamin E deficiency may be due to peroxidation of lipids of cellular mitochondria.*

Although the factor known as vitamin E has been studied for 40 years, the mechanism of its action and indeed its effects on man remain poorly understood. The characteristics of vitamin E deficiency in animals have been described repeatedly. These include retardation of growth of the young, infertility of adults, and the development of myopathies of both skeletal and cardiac muscle. In addition, encephalomalacia, degeneration of the kidneys, and several other peculiar syndromes have been observed (*Nutrition Reviews* **17**, 60, 154 (1959)). Animals with muscular dystrophy may have ceroid deposits in their muscular tissue and they

excrete abnormal quantities of creatine in their urine.

Muscle from deficient animals has an increased rate of oxygen utilization (O. B. Houchin and H. A. Mattill, *J. Biol. Chem.* **146**, 309, 313 (1942)). This observation led to the discovery that vitamin E has an antioxidative effect. The erythrocytes of deficient animals can be hemolyzed more readily than normal by dilute solutions of hydrogen peroxide (C. S. Rose and P. György, *Am. J. Physiol.* **168**, 414 (1952)).

If human deficiency of vitamin E is to occur spontaneously it must happen in persons who have some absorptive defect

or in those who are fed an unusual diet, since the vitamin is both widely distributed and readily absorbed. These are precisely the circumstances present in premature infants, whose absorptive capacities are incompletely developed and who are usually given a diet composed primarily of dilute skimmed milk and carbohydrate.

For this reason, R. B. Goldbloom (*Canad. Med. Assn. J.* **82**, 1114 (1960)) studied the concentration of tocopherols in the serum of infants and children in comparison with that of adults. He also measured erythrocyte survival of chromium-51 tagged cells in laboratory animals and in one subject in an attempt to find a hemolytic process. He measured the tocopherol content of serum from healthy adults, normal premature infants, full-term infants less than seven days old, healthy children between the ages of two and 13 years, children with biliary atresia and patients with fibrocystic disease of the pancreas.

Normal adults had concentrations consistently above 0.5 mg. per cent with a mean value of 0.77 mg. per cent, and normal children had similar values. Healthy infants, premature infants and those with biliary atresia had values of about 0.2 mg. per cent. Patients with fibrocystic pancreatic disease had the lowest average values (0.11 mg. per cent).

Erythrocyte survival studies in vitamin E-deficient rats and in a two-year-old boy with fibrocystic disease of the pancreas (tocopherol level of 0.02-0.26 mg. per cent) were quite normal.

These findings led the author to consider that premature infants and children or adults with absorptive defects might benefit from the addition of tocopherol to their diet. The author is conducting an additional study of the effects of supplementing the diet of premature infants, the results of which should be of considerable interest.

It seems particularly helpful to learn that deficiency of vitamin E did not cause erythrocytic hemolysis despite the fact that

these red cells are more susceptible to hydrogen peroxide. This observation would have been of much greater significance had the author determined hydrogen peroxide fragility of the blood specimens.

Aside from the antioxidant effect of vitamin E, very little is known regarding its mechanism of action. In a separate article, H. Zalkin and A. L. Tappel (*Arch. Biochem. Biophys.* **88**, 113 (1960)) reported evidence for postulating that this vitamin acts by preventing lipid peroxidation of mitochondria. They fed a vitamin E-deficient diet to white rabbits until muscular weakness developed. Then they sacrificed the animals along with healthy controls and studied the thiobarbituric acid reaction of their tissues. (This reaction measures malonaldehyde, which is formed by lipid peroxidation, and can be used to measure peroxidation of tissues and of mitochondria.) In deficient animals they found significantly more thiobarbituric acid reactant in the kidneys and in the liver than in healthy animals.

Their study further sought to determine whether the alterations of the mitochondria might affect oxidative phosphorylation. It was found that this function was impaired with regard to phosphorylation of succinate but not with regard to beta-hydroxybutyrate or alpha-ketoglutarate.

The demand for vitamin E can be met by supplying animals with other antioxidants (*Nutrition Reviews* **17**, 174 (1959)), and yet not all of the actions of this vitamin are duplicated by such compounds (*Ibid.* **17**, 340 (1959); **18**, 193 (1960)). Zalkin and Tappel discussed the comparative effects of vitamin E deficiency and those of ionizing radiation. At least part of the toxicity of such radiation may depend upon production of lipid peroxides (V. J. Horgan *et al.*, *Biochem. J.* **67**, 551 (1957)). Since lipid peroxidation may result in such free radicals as lipid peroxy, lipid oxy, lipid hydroxy, and others resulting from cleavage of unsaturated fatty acids, these products might

be expected to react with and inactivate a variety of compounds including enzymes and vitamins (Tappel, *Arch. Biochem. Biophys.* **50**, 473 (1954)). Much of the unsaturated fat of an animal cell resides in the mitochondria in close proximity to the cytochromes, and the latter contain some of the most potent catalysts of lipid peroxidation (Tappel, *Ibid.* **44**, 378 (1953)).

Thus in their present report, Zalkin and Tappel considered that the antioxidant effect of vitamin E could explain all the complicated metabolic functions of this vitamin.

Such studies may lead to a sufficient understanding of the action of vitamin E so that its place in clinical medicine may become firmly established.

## NIACIN AND STEROL METABOLISM

*High levels of niacin, niacinamide, isonicotinic acid and benzoic acid were fed to rats and chicks. Blood cholesterol levels in chicks were depressed by niacin, and in both rats and chicks by isonicotinic acid.*

Niacin when fed in large doses to man effectively reduces plasma cholesterol levels in hypercholesterolemic patients, according to the report of W. B. Parsons, Jr., and J. H. Flinn (*Arch. Int. Med.* **103**, 783 (1959); See also *Nutrition Reviews* **17**, 78, 168, 291 (1959)). These authors found, however, that niacinamide does not lower blood cholesterol appreciably in man (*Circulation* **16**, 499 (1957)).

In order to investigate the mechanism by which blood cholesterol is reduced by niacin, J. L. Gaylor, R. W. F. Hardy and C. A. Baumann (*J. Nutrition* **70**, 292 (1960)) studied the effects of niacin and niacinamide as well as related compounds in rats and chicks. Blood and liver cholesterol were measured along with liver fat and pyridine nucleotides and fecal fatty acids, sterols and bile acids.

Male albino rats weighing 100 g. were fed diets containing from 0.1 to 1.0 per cent niacin, niacinamide or isonicotinic acid, and 1.0 per cent benzoic acid or 2 per cent tryptophan. The basal diet contained 18 per cent casein, 67 per cent sucrose, 10 per cent lard and adequate salts and vitamins. The chick basal diet contained in per cent: casein, 23; sucrose, 56; gelatin, 10; salts, 6; soybean oil, 4; and

adequate vitamins. One per cent of niacin, niacinamide, isonicotinic acid or benzoic acid was added to the basal diet.

In the rat experiments, three groups of 12 rats were fed a diet containing 1 per cent cholesterol and 0.5 per cent cholic acid in order to elevate the blood cholesterol level, which increased from 100 mg. per cent to an average of 203 mg. per cent in two weeks. Then one group was continued on the original diet and the other two groups were given supplements of 0.2 and 1.0 per cent of niacin, respectively.

The cholesterol level rose steadily in all groups, but the rise was less rapid in the rats receiving the 1.0 per cent of niacin. Peak values at four weeks averaged 397 mg. per cent for controls, 340 mg. per cent for the 0.2 per cent niacin group and 450 mg. per cent for the group fed 1.0 per cent niacin. Group averages at seven weeks were 297, 306 and 292 mg. per cent. This indicates that, under these conditions, blood cholesterol in hypercholesterolemic rats does not respond to niacin therapy.

Since the above diet does not resemble the type of human diet in which niacin is usually effective, new groups of rats were given the basal diet containing 10 per cent lard as the only source of cholesterol.

Supplements were 0.1 and 1.0 per cent of niacin, niacinamide or isonicotinic acid, 2 per cent tryptophan and 1 per cent benzoic acid. The ad libitum consumption of the diet containing niacin resulted in significant increases in free and total cholesterol. Free cholesterol also increased with 2.0 per cent tryptophan feeding. Neither niacinamide nor benzoic acid changed the cholesterol level, but isonicotinic acid tended to reduce it.

In the chick experiments, both male and female chicks were fed ad libitum either the basal chick diet alone or supplemented with 10 per cent of lard or with lard plus 0.5 per cent cholesterol replacing the soybean oil and some sucrose. After two and four weeks, blood cholesterol was determined. The addition of cholesterol to the diet doubled the blood cholesterol level, the increase being mostly in ester cholesterol. After two weeks, the addition of 1 per cent niacin depressed the cholesterol significantly and the effect persisted at four weeks. Although niacin appeared to depress blood cholesterol in chicks fed lard with no added cholesterol, the differences were not significant. Only slight lowering was observed with niacinamide.

Blood cholesterol levels of chicks eating the basal soybean oil ration were significantly reduced by 1 per cent niacin or isonicotinic acid, but not significantly affected by niacinamide or benzoic acid.

Determination of pyridine nucleotide contents of livers showed a 100 per cent rise in rats and less in chicks upon feeding niacin, and a much greater increase when niacinamide was given. This suggests that the effectiveness of niacin in reducing blood cholesterol is not due to a greater conversion to pyridine nucleotides.

Liver fat and cholesterol were determined on rats and chicks fed the basal diet with and without niacin and related supplements. They were also measured following the feeding of rations containing lard

or lard plus cholesterol and cholic acid, with and without niacin and related compounds. Cholesterol supplementation elevated the control liver cholesterol in rats from 3.8 mg. per g. of dry weight to over 21 mg. per g., and raised control values in the chick from 7 mg. per g. to over 26 mg. per g. Under these conditions, neither niacin nor niacinamide lowered liver cholesterol. In fact, substantial elevation occurred in the chicks.

Benzoic acid, niacin and niacinamide increased liver fat in the absence of added cholesterol in the rat, and isonicotinic acid lowered liver fat in both the rat and chick.

Liver cholesterol in chicks fed the ration with added cholesterol averaged 26.4 mg. per g. dry weight, and this was increased to 40.9 mg. per g. upon niacin supplementation and to 38.7 mg. per g. upon addition of niacinamide. The livers of rats and chicks fed the basal diets showed no change in liver fat or cholesterol after being fed niacin and related compounds.

In rats, a depressed growth rate followed the feeding of niacinamide, and niacinamide plus 2 per cent tryptophan reduced the growth rate to about 40 per cent of the control. However, neither niacin nor isonicotinic acid depressed growth. In chicks, growth was reduced by niacin from 8 to 17 per cent below that of control animals. A somewhat greater depression occurred with niacinamide and benzoic acid, while isonicotinic acid had no effect.

Measurement of fecal bile acids and fatty acids showed no alteration as a result of niacin feeding. Niacinamide increased the amount of fecal fatty acid and isonicotinic acid increased the bile acid excretion. Apparently the cholesterol-lowering effect of isonicotinic acid occurs in some metabolic event prior to and in preference to increased excretion of bile acids.

It is obvious from these studies that isonicotinic acid is by far the most con-

sistently effective and the least toxic of the drugs investigated.

Its effect on experimental lesions should

certainly be studied. It seems likely that it may produce its effect by increasing the catabolism of cholesterol.

### INHIBITION OF "BROWNING REACTION"

*Inhibitors are described for the chromogenic reaction between glucose or fructose and fibrin hydrolysates. Fructose solutions with fibrin hydrolysates lose nutritive value less rapidly during storage than do glucose solutions.*

Foodstuffs containing mixtures of amino acids and reducing sugars may deteriorate in quality because of the interactions of these two substances to form brown pigments. J. E. Hodge (*J. Agr. Food Chem.* **1**, 928 (1953)) has described this "browning reaction" in detail. It has further been shown that the nutritive value of fibrin hydrolysates with dextrose is related inversely to the color of the solution (L. R. Overby and D. V. Frost, *J. Nutrition* **46**, 539 (1952)). Subsequently, various sulfur-containing antioxidants were found to inhibit the development of color and delay loss of amino acid food value.

Recently, Overby, R. L. Fredrickson and Frost (*J. Nutrition* **69**, 318 (1959)) compared potassium metabisulfite, sodium hydrosulfite and sodium formaldehyde sulfoxylate with respect to their capacity for inhibiting color formation in fibrin hydrolysate or glycine solutions with added glucose or fructose. Loss of nutritive value of the solutions was assayed by rat repletion.

The fibrin hydrolysate was a commercial preparation from beef fibrin containing about two-thirds free amino acids and one-third small peptides. Four solutions were prepared: (a) 80 g. of glycine and 200 of glucose diluted to 2000 ml. with water; (b) a similar solution of 80 g. of glycine and fructose; (c) 200 g. of glucose dissolved in a quantity of fibrin hydrolysate so that, when diluted to 2000 ml., the total nitrogen was 6.5 mg. per ml; and (d) a similar solution of 200 g. of fructose with fibrin hydrolysate.

The solutions were divided into 500 ml. portions and placed in closed bottles under carbon dioxide atmosphere. To three bottles of each of the four solutions one of the sulfur-containing inhibitors was added. All solutions were then autoclaved at 110° C for 20 minutes; a sample was then removed and the optical density (o.d.) measured at 385 m $\mu$ . The following optical densities were found in the four solutions autoclaved without inhibitors: fibrin hydrolysate with glucose, 1.8; fibrin hydrolysate with fructose, 0.830; glycine with glucose, 0.449; glycine with fructose, 0.790.

With fructose-fibrin hydrolysate, sodium formaldehyde sulfoxylate was the most effective inhibitor (o.d. = 0.323) followed by potassium metabisulfite (o.d. = 0.340) and sodium hydrosulfite (o.d. = 0.430). With fructose-glycine solutions, practically the reverse picture was observed; sodium formaldehyde sulfoxylate was least effective (o.d. = 0.251), sodium hydrosulfite was better (o.d. = 0.190), and potassium metabisulfite was best (o.d. = 0.035), but the degree of "browning" was less in all cases than with fibrin hydrolysate.

The order of effectiveness of the inhibitors with glucose-glycine and glucose-fibrin hydrolysate was not different from that found with fructose solutions, but all glucose-fibrin hydrolysate solutions showed more color and glucose-glycine solutions less color than the corresponding solutions with fructose.

For the nutritional studies, solutions of



fibrin hydrolysate with glucose or fructose were prepared as above with sodium formaldehyde sulfoxylate. Following sterilization at 110° C for ten minutes, they were either used immediately or further treated in one of the following ways before testing: (a) stored at 25° C for 15 months; (b) stored at 4, 25, and 40° C for four and a half months; (c) autoclaved 15 minutes at 121° C; and (d) autoclaved 30 minutes at 121° C.

Nutritive values of the "browned" solutions were determined by the rat repletion method of Frost and H. R. Sandy (*J. Biol. Chem.* **175**, 635 (1948)). Rats on a diet of fibrin hydrolysate with 10 per cent glucose showed a gain of 6.1 g. in 12 days. Those on the 10 per cent fructose gained an average of 15.6 g. and those on a control solution of fibrin hydrolysate lacking carbohydrate gained an average of 19.8 g.

Fibrin hydrolysate with fructose after storage at 40° C for four and a half months showed about a 10 per cent loss in nutritive

value by actual weight gain, but this was not statistically different from the weight gains of the other three groups. When an accelerated rate of browning was produced by heat treatment, it was found that 121° C for 15 minutes caused little or no loss of nutritive value, whereas 30 minutes exposure to this temperature resulted in a definite loss of nutritive value of the fibrin hydrolysate.

The authors point out that the three sulfite compounds employed in these experiments represent three different levels of oxidation-reduction potential. The least highly oxidized of these compounds, sodium formaldehyde sulfoxylate, was the most potent inhibitor in the case of the complex mixture of fibrin hydrolysate-carbohydrate. However, the model system glycine-carbohydrate was protected best by the most highly oxidized compound, bisulfite. No explanation for these seemingly contradictory results is offered.

## CHOLESTEROL AND ESSENTIAL FATTY ACID DEFICIENCY

*Cholesterol added to the ration of weanling male rats accelerated the development of essential fatty acid deficiency.*

Attempts to accelerate the production of essential fatty acid deficiency led J. J. Peifer and R. T. Holman (*Arch. Biochem. Biophys.* **57**, 520 (1955)) to try the stress of alloxan diabetes. They reasoned that since catabolism and mobilization of fat is accelerated in diabetes it could result in the concomitant raiding of essential fatty acid stores. Feeding weanling rats an essential fatty acid (EFA)-free diet and making them diabetic by injecting alloxan resulted in the development of severe EFA deficiency symptoms within one month.

Several facts suggest the possibility that cholesterol metabolism could be involved in the development of this EFA deficiency. Hypercholesteremia is associated with diabetes. Essential fatty acids are present in

cholesterol esters and cholesterol esters tend to accumulate in the liver during the development of essential fatty acid deficiency. Thus the normal transport of cholesterol may depend on the presence of adequate supplies of EFA. For these reasons, Holman and Peifer (*J. Nutrition* **70**, 411 (1960)) have studied the effects of feeding cholesterol along with hydrogenated coconut oil (HCO) on the deficiency of EFA.

Weanling male rats 21 days old were fed a fat-free diet for a week before being distributed at random into four groups eating diets with the following supplements: group one received 1 per cent hydrogenated coconut oil (HCO), group two received 1 per cent HCO and 1 per cent

cholesterol, group three received 1 per cent corn oil ethyl esters and group four received 1 per cent corn oil ethyl esters plus 1 per cent of cholesterol.

Within five weeks EFA deficiency appeared. The dermal score was more pronounced in animals receiving 1 per cent cholesterol. Growth was inhibited, food efficiency was diminished and the development of the testes was inhibited. Dietary cholesterol given to rats fed EFA rather than acting as a stress resulted in improved food efficiency, body weight gain and testes weight increase. Severe dermal symptoms normally appearing after three months of a fat-free diet regimen were manifest only five weeks following cholesterol supplementation.

Deficiency of essential fatty acids results in histological changes in rat testes, according to the report of T. C. Panos and J. C. Finerty (*J. Nutrition* **54**, 315 (1954)). If dietary cholesterol is used to hasten the depletion of essential fatty acids, severe degeneration occurs in spermatogenic tissue, according to Holman and E. Aaes-Jorgensen (*Proc. Soc. Exp. Biol. Med.* **93**, 175 (1956)). In the present experiment, rats fed an ESA-free diet had significantly lower testis weights than those fed EFA. Marked atrophy occurred in testes of some rats fed cholesterol and EFA-free diet.

Qualitative studies of essential fatty acid deficiency involved grading of the severity of dermal signs of deficiency. Groups of eight to ten 21-day-old weanling male rats were fed various combinations of diets with or without hydrogenated coconut oil (HCO), linoleate or cholesterol.

Group one fed a basal diet containing 1 per cent linoleate did not develop appreciable dermal signs. Groups two, three and four developed severe dermal signs after four weeks on a diet containing 1 per cent HCO and 1 per cent cholesterol. At the end of four weeks group two was fed 1 per cent linoleate without cholesterol and group three was fed 1 per cent linoleate with 1

per cent cholesterol, while groups one and four were continued on their original diets. The dermal symptoms of groups two and three improved equally, indicating the availability of EFA even in the presence of dietary cholesterol.

Group five fed 1 per cent cholesterol and 1 per cent linoleate did not show dermal signs of deficiency. Group six was fed 1 per cent HCO and 1 per cent cholesterol during the first four weeks. During the next three weeks cholesterol was removed but deficiency symptoms continued to become more severe. Groups seven and eight fed 1 per cent HCO plus 2 and 5 per cent cholesterol did not develop more severe symptoms than animals receiving less cholesterol, probably because these amounts exceeded the capacity of the rat intestine to absorb cholesterol as reported by T. M. Lin, E. Karvinin and A. C. Ivy (*Proc. Soc. Exp. Biol. Med.* **89**, 422, (1955)).

During their studies of fatty acid deficiency, Holman and Peifer observed a great variation in the length of time needed to produce deficiency symptoms in 21-day-old weanling rats. Since the time varied from 20 to 60 days they were prompted to try the use of younger animals. After facing some problems of high mortality they were able to use 15-day-old rats in producing more severe EFA deficiency rapidly and uniformly.

Study of the polyenoic fatty acids of heart tissue was made in an effort to quantitate the effects of cholesterol on EFA deficiency. Various diets were fed the 15-day-old weanling male rats for 43 days. Analysis of the hearts showed that polyenes of EFA-deficient groups tended to accumulate in heart muscle, the largest accumulation being in the group fed 1 per cent HCO plus 1 per cent cholesterol. Trienes predominated while tetraenes represented a smaller proportion of total polyenes. Feeding of linoleate decreased the per cent of trienes and increased the per cent of tetraenes.

Total polyunsaturated fatty acids in-

creased in heart tissue of EFA-deficient rats. Since there are no dietary precursors, this increase probably represents mobilization rather than synthesis. The presence of extra cholesterol in the animal may result in an increased need for EFA in order to facilitate transfer of cholesterol.

Further work along this line may help to establish biochemical mechanisms by which

relative EFA deficiency might lead to lipid accumulation in blood vessels. Whether this is a facet of lipoprotein structure and metabolism remains to be investigated. If cholesterol is transported and ultimately metabolised as a part of a lipoprotein complex, it may be that EFA may act mainly to regulate lipoprotein synthesis or catabolism.

## HORMONAL FACTORS IN LIPID MOBILIZATION

*Epinephrine and norepinephrine mobilize fat from adipose tissue in vitro. Purified corticotrophin does so without mediation of the adrenal glands.*

Research on fat metabolism has excited much renewed interest since the demonstration by isotope techniques that fatty acids have an extremely rapid turnover in the body (*Nutrition Reviews* 17, 241 (1959)) and that adipose tissue has a very active metabolism (*Ibid.* 17, 280 (1959)). A number of physiologic substances, some long-known, affect the mobilization of depot fat. An earlier article on this subject (*Ibid.* 13, 207 (1955)) discussed the role of local innervation of adipose tissue, C. H. Best's and J. Campbell's "adipokinin" from the anterior pituitary, and the specific pituitary and adrenal hormones. A more recent review discusses adrenals and fat mobilization (*Ibid.* 17, 246 (1959)).

Suitable analytic techniques have permitted *in vitro* study of fragments of adipose tissue with measurement of various metabolites in the incubation medium under different conditions. "Lipolysis", or lipid mobilization from adipose tissue, now proves quite amenable to study and we are presented with a fascinating array of findings: epinephrine mobilizes lipid (see below), corticotrophin does so, but not through the adrenals (see below), and growth hormone has a similar effect. There is the lipid-mobilizing hormone of J. Seifter and D. H. Baeder, perhaps of pituitary origin (*Nutrition Reviews* 18, 275 (1960)). D. Rudman's

lipemia-producing hormone from the pituitary has quite different action (*Ibid.* 18, 297 (1960)). Thus the pituitary may secrete several substances affecting mobilization of depot fat.

J. E. White and F. L. Engel (*Proc. Soc. Exp. Biol. Med.* 99, 375 (1958)) studied the lipolytic action of epinephrine and norepinephrine on rat adipose tissue *in vitro*. They were led to this study because epinephrine injection in man causes an increase in serum nonesterified fatty acids. The promptness of the rise suggested that the effect was directly on adipose tissue; this had been shown to be true *in vitro* by the authors and others.

White and Engel incubated portions of epididymal adipose tissue from fasting rats in heparinized pooled rat plasma or serum at 36°C, with epinephrine or norepinephrine (solutions of L- or D- salts). Incubation for three hours without hormone caused no change in the fatty acid content of the medium and a decrease (due to artifact) in the fatty acid content of the tissue. However, 5 µg. of L-epinephrine caused a "striking" increase within three hours in the fatty acid content of the medium and in the adipose tissue. Exposure to 10 µg. of L-epinephrine for 15 minutes produced twice as much tissue fatty acid concentration as in tissue incubated without hormone; within

that time the plasma level did not change appreciably, suggesting that the new fatty acids were formed in the adipose tissue and not the plasma.

Incubation under nitrogen reduced considerably the epinephrine effect in medium and in tissue. The fatty acid content of the medium increased in the hormone-free controls under nitrogen, but not in air. White and Engel, therefore, infer that aerobic metabolism is required for the lipolytic action of epinephrine.

L-Epinephrine and L-norepinephrine were equally active and 100 to 200 times as potent as the respective D-isomers.

The authors conclude that epinephrine and norepinephrine probably stimulate hydrolysis of neutral fats directly in adipose tissue. The inactivity of the unphysiologic D-isomers strengthens their belief that the tissue response is a specific one.

Both corticotrophin and growth hormone produce fatty liver and ketonemia. White and Engel (*J. Clin. Invest.* **37**, 1556 (1958)) found that they both had lipolytic effects *in vitro*.

These authors, in another paper entitled "Fat mobilization by purified corticotrophin in the mouse", have shown that this action is not adrenal-mediated (*Proc. Soc. Exp. Biol. Med.* **102**, 272 (1959)). They removed one testis from etherized fasting mice and dissected away the epididymal fat pad. Hormones were injected intraperitoneally and the animals allowed to recover for six hours; water was allowed, but no food. The liver and second fat pad were then removed and the total extractable lipids in the two pads were compared. Mice adrenalectomized at the time the first fat pad was removed were maintained for the next six hours on 0.9 per cent saline solution.

Both growth hormone and corticotrophin (ACTH) caused a significant decrease in fat pad lipid (17.3 and 20.8 per cent respectively), but hydrocortisone did not. There was little change in liver fat. Fat pads of mice adrenalectomized when the first fat

pad was removed showed fat loss (7.9 per cent). ACTH did not increase this significantly, and this weakens the authors' conclusions. However, when given together with hydrocortisone, it overcame the inhibition of lipolysis induced by the latter.

The authors infer, from these experiments and earlier *in vitro* work, that both growth hormone and corticotrophin act directly on adipose tissue depots. The lipolytic effect of growth hormone is much less than that of ACTH in equally ketogenic doses, and presumably not due to contamination. Although the importance of epinephrine and norepinephrine in this respect is not established, White and Engel infer that increased ACTH secretion secondary to adrenalectomy may account for the greater epididymal fat loss in adrenalectomized mice (7.9 per cent) than in the intact animals (2.5 per cent). They point out that hydrocortisone has no inhibitory effect *in vitro* and may well act *in vivo* by inhibiting ACTH secretion. The role of other pituitary hormones or of insulin was not studied.

Another of the accumulating reports on the *in vitro* endocrinology of adipose tissue is that of B. Leboeuf, R. B. Flinn and G. F. Cahill, Jr., entitled "Effect of epinephrine on glucose uptake and glycerol release by adipose tissue *in vitro*" (*Proc. Soc. Exp. Biol. Med.* **102**, 527 (1959)).

These authors show that the release of fatty acids from adipose tissue is not necessarily linked to carbohydrate utilization. Although an inverse relationship obtains in many situations (*i.e.*, diabetes mellitus), they point out that epinephrine causes both glucose uptake and fatty acid release by adipose tissue *in vitro*.

Leboeuf, Flinn and Cahill incubated fragments of epididymal fat pads from fed rats in a buffer containing human serum albumin and glucose. The glucose, glycerol and fatty acid contents of the medium were determined after incubation for three hours with and without the addition of hormones.

Glucose uptake was increased by insulin and by epinephrine when compared to incubated controls. Free fatty acid was taken up slightly in the controls and with insulin but released by epinephrine. Likewise, glycerol release was slight in the controls, slightly less with insulin and considerable with epinephrine.

The authors point out that epinephrine inhibition of peripheral glucose utilization *in vivo* and in muscle incubated *in vitro* is probably attributable to glycogenolysis and that the virtual absence of glycogen from adipose tissue may account for the observed

differences. They speculate as to why insulin and epinephrine have similar effects on glucose uptake but opposite effects on the release of free fatty acids, and conclude that epinephrine probably mobilizes fatty acids by increased glyceride breakdown rather than by decreasing carbohydrate availability.

The hormonal factors in lipid mobilization are evidently many, and their actions and interrelationships incompletely known. *In vitro* studies such as those reviewed above provide a means of extending our knowledge considerably.

### VITAMIN A ACID AND THE FUNCTION OF VITAMIN A

*Vitamin A acid maintains growth and general health of rats but cannot be stored and does not prevent night blindness or retinal changes.*

In a recent study, J. E. Dowling and G. Wald, *Proc. Nat. Acad. Sci.* **44**, 648 (1958); *Nutrition Reviews* **17**, 22 (1959)) investigated the course of vitamin A deficiency in rats. From the results, the authors concluded that vitamin A (as the aldehyde, retinene) is not only involved with prevention of night blindness but also with protection of the protein, opsin, and the rods, which deteriorate in severe vitamin A deficiency. Indeed, it was suggested that the general effect of this vitamin, the lack of which results in the disintegration of epithelial and other tissues, might lie in a similar protection of structural proteins in these tissues by combination with some derivative of vitamin A.

Continuing the study of this exciting possibility, Dowling and Wald (*Proc. Nat. Acad. Sci.* **46**, 587 (1960)) investigated the effect of vitamin A acid on vitamin A-deficient rats. This acid, which is identical in structure to vitamin A except for the oxidation of the primary alcohol of the latter to a carboxyl, had previously been shown by J. F. Arens and D. A. Van Dorp (*Nature* **158**, 622 (1946)) to prevent obvious signs of vitamin A deficiency without contributing any vitamin A to liver storage.

Several different types of experiments were performed:

To test the effect of vitamin A acid on growth and general condition, weanling rats were raised on an otherwise adequate vitamin A-deficient diet with or without supplementation with vitamin A acid (at 50  $\mu$ g. per day) or vitamin A alcohol. The animals without the supplements grew normally for about 40 days, then stopped growing and died at about 60 days. Those with the vitamin A acid supplement grew about as rapidly as did those with vitamin A alcohol and, except for visual difficulties, appeared perfectly normal to external examination. Moreover, the acid was immediately effective in restoring growth of depleted animals. It was evident, therefore, that for growth and general health the acid was as effective as the alcohol.

To investigate the possible conversion of vitamin A acid to the alcohol, liver stores of rats maintained on the acid were analyzed. It was found that liver vitamin A declined as rapidly in the animals with vitamin A acid supplement as in those on the deficient diet alone. Moreover, there is apparently

no storage of the acid since, when the acid supplement was removed, rats apparently in good condition stopped growing and became severely deficient in a few days. In normally nourished adult animals, large stores of vitamin A usually maintain the animals for long periods after removal of the vitamin from the diet. Sensitive chemical tests for vitamin A acid also failed to reveal the presence of any of the acid in livers of rats receiving this supplement and thus confirmed the results of the growth studies.

It is evident, then, that the vitamin A acid is very rapidly destroyed and that it cannot be reduced to the aldehyde or alcohol.

The results of a study of the visual thresholds of rats on the acid-supplemented diet are of particular interest. In the unsupplemented rats dying at about 60 days, moderately severe night blindness had developed with visual thresholds about 100 times normal (the visual threshold is the smallest luminance of a  $\frac{1}{50}$  second flash light needed to evoke a just perceptible electroretinogram (ERG)). The rats with the vitamin A acid supplement developed night blindness at the same rate but since they were otherwise in good condition, continued to show higher visual thresholds until at about 140 days they had leveled off at about 2000 to 3000 times normal. At this time, the retinal rhodopsin was found to be only 1 to 5 per cent of normal. When the diet was continued, it was found that visual stimuli even 10,000 times the threshold level failed to yield a normal ERG response, and after ten months the animals were completely blind.

Examination of the retinas of the deficient animals with the electron microscope revealed that, as the visual threshold increased, the visual cells decreased and the outer segments of the rods began to disintegrate. After ten months, only a single row of nuclei remained of the visual cells and

both inner and outer segments of the rods had disappeared.

When vitamin A alcohol was administered to animals that had been on the acid supplementation for different lengths of time, varying degrees of recovery were obtained. Up to ten weeks, recovery was rapid and complete. At 25 weeks, a two phase recovery was seen: a rapid recovery to visual thresholds about ten times normal and a much slower return to normal. The authors concluded that the first stage represents combination of retinene with the opsin of the remaining rods, while the slower stage was for resynthesis of opsin and reformation of lost rods.

After ten months, recovery could not be elicited and the visual cells had apparently been lost or had dedifferentiated to the extent that they could no longer form outer segments.

The authors have come to several significant conclusions:

First, vitamin A alcohol (the transport form of the vitamin and the storage form, as the ester) is oxidized to retinene in the retina, in spite of an unfavorable equilibrium, because of the rapid removal of the aldehyde in its combination with opsin to form rhodopsin. This is apparently the only function of the aldehyde. The alcohol is also oxidized to the acid, presumably in the liver, and the latter is either rapidly converted to an active product or is rapidly used in its functions of growth and tissue maintenance. In any event, the acid (or its product) may be the important form of vitamin A in its general function, but it must be stored as the alcohol and cannot be reconverted to either the aldehyde or alcohol.

With these experiments, another step has been taken in the understanding of the functions of vitamin A. It is to be hoped that information on the exact mechanism by which vitamin A acid may act in maintaining epithelial tissue will be shortly forthcoming.



## NOTES

## Letters to the Editor

Sir:

In a special article (*Nutrition Reviews* 15, 257 (1957)) D. M. Hegsted indicated the need for a re-examination of the situation concerning the present high recommended intakes of calcium. Supporting his view (*Ibid.* 16, 31 (1958)), I questioned the wisdom of recommending luxury intakes of the element in the absence of specific deficiency stigmata.

In a recent study on pathological grading and chemical composition of aorta from Bantu and white subjects, we drew attention to the far higher concentration of calcium in white compared with Bantu aorta from middle age onwards, and even in aorta of equal pathological grading (M. Anderson *et al.*, *A. M. A. Arch. Path.* 68, 380 (1959)). In a paper just published, A. E. Hirst Jr., *et al.* (*Arch. Path.* 69, 578 (1960)) also have noted the far greater incidence and severity of calcification lesions in aorta from Los Angeles whites compared to Indians in South India. Both the latter population and the South African Bantu have low calcium intakes, lesser severity of aortic and coronary lesions, and a much lower mortality from coronary heart disease.

The precise bearing of arterial calcification on mortality from coronary heart disease is not known. But if, to ward off death from this cause, a reduction in the amount of fat consumed is recommended, would it not be logical also to recommend a modicum of reduction in the present high calcium intakes ingested by more privileged populations?

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Dear Sir:

The review of the effect of antioxidant

on vitamin E deficiency in chicks (*Nutrition Reviews* 17, 340 (1959)) prompts the following comments which may be useful, since much of our work was not submitted to journals normally read by many investigators in animal nutrition.

In all species tested to date (man: *Am. J. Clin. Nutrition* 4, 408 (1956); 8, 451 (1960); *A. M. A. Arch. Neurol.* 1, 312 (1959); the chick: *Proc. Soc. Exp. Biol. Med.* 102, 375 (1959); *A. M. A. Arch. Gen. Psychiat.* 1, 420 (1959); the rat: *J. Nutrition* 72, Nov. issue (1960)), the level of alpha-tocopherol required to inhibit reactions attributable to vitamin E deficiency were functions of the amounts of peroxidizable compounds present in the diet or in the tissues. For example, little if any added tocopherol is required when only small amounts of polyunsaturated fatty acids are present, but the requirement for tocopherol increases markedly as the level of linoleic acid in the diet is increased.

Accordingly, one should never interpret an experiment on tocopherol need without evaluating how much total polyunsaturated fatty acid is in the diet. It should be emphasized that even lard, which normally contains about 10 per cent linoleic acid (and much more if the pig was fed unsaturated fats), can provide as much linoleic acid at a 20 per cent level in the diet as corn oil at a 4 per cent level. Both of these levels of fat are sufficient to produce encephalomalacia in most chicks deprived of tocopherol since they provide more than 2 per cent linoleic acid in the diet.

The fatty acid profile of all of the tissue lipoproteins and phospholipids tested today are influenced by the levels of various fatty acids in the lipids consumed, either from the diet or as a result of previous ingestion. The effect of diet on tissue lipid composition, which hog raisers have con-

sidered to be important for many years, is just as important in determining the fatty acid composition of brain mitochondria and erythrocytes (*Science* **130**, 917 (1959)). The types of pathology noted during vitamin E deficiency may be related to the rates of growth (lipid accumulation), the relative alterations in the polyunsaturated fatty acid composition of the organs affected, and whether or not there is adequate antioxidant at the site of the enzymes or other microconstituents of the cells in question.

The means by which changes in fatty acid composition can affect physiological function is not yet clear, but a recent report by F. D. Collins (*Nature* **186**, 366 (1960)) showing that the more unsaturated lecithins from rat livers took up more  $P^{32}$  should be considered in the interpretation of these effects.

Presumably, other lipid-soluble antioxidants could function as well as alpha-tocopherol if they were completely nontoxic and if they could be absorbed and directed to the specific intracellular sites that need protection, but to date no complete substitute for alpha-tocopherol has been found. Since the cellular absorption and excretion rates of synthetic antioxidants can be expected to be different from those of alpha-tocopherol, one should not be surprised to obtain different physiological effects when they are used. Such differences do not prove that vitamin E has additional functions other than that of an antioxidant.

Although some chemical signs of lipid peroxidation, such as increases in "ceroid" pigment, are not difficult to demonstrate either as a function of acute vitamin E deficiency or as a consequence of long exposure to accumulated oxidations, as in aged animals, the demonstration of specific peroxidized lipids in tissue has not been entirely satisfactory. Unfortunately, the thiobarbituric acid test for peroxides is in part a function of the amount of tocopherol and the amounts of peroxidizable lipids present in tissue, and the technique as it is

usually conducted measures amounts of peroxide formed during the procedure as well as the amounts of preformed peroxide products. More work needs to be done to prove that peroxides are formed in tissue since it is logical that they should be present during vitamin E deficiency.

Vitamin E may have antioxidant functions other than that of protecting polyunsaturated fatty acids. Thus it may be useful in protecting vitamin A, certain sterols and other tissue constituents not now recognized. In the above discussion, selenium is considered to be supplied adequately and is not a part of this antioxidant story, but the possibility remains that on certain special diets there is need for antioxidant protection of some unknown vital selenium compound or of other metallo-organic complexes.

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### Recent Books

*Food for Space Travel*. By Albert A. Taylor, Beatrice Finkelstein and Robert E. Hayes. Air Research and Development Command Technical Report No. 60-8, Andrews Air Force Base. Copies may be obtained from the Office of Technical Services, U. S. Department of Commerce, Washington 25, D. C. Pp. 64.

*Manual of Nutrition*, Fifth Edition. Prepared by the Ministry of Agriculture, Fisheries and Food and the Central Office of Information. Published by Her Majesty's Stationery Office, London. 1959. Pp. 72. Price 3 shillings net.

### Erratum

The italicized summary of the review "Scurvy and Blood Coagulation" appearing on page 242 of the August 1960 issue should have stated that ascorbic acid deficiency resulted in an "increase in platelets" rather than a "reduction".

# NUTRITION REVIEWS

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## INTRODUCING NEW FOODS AGAINST PROTEIN DEFICIENCY

Human beings have changed their food habits in the past and will continue to do so in the future. The rather pessimistic view taken by some workers of the impossibility of changing the food habits of developing or underdeveloped communities is not supported by the facts. Protein malnutrition is highly prevalent in many parts of the world, and, naturally enough, most of the efforts of nutritionists, educators, governments and agricultural experts are directed towards finding foods which may prevent protein malnutrition.

In many of the affected areas milk and milk products are not available to the general population and meat and fish find their way into the diet more as flavoring agents than as nutritive components. Nor is there any immediate possibility of these good sources of protein being produced in sufficient quantity and cheaply enough to be used in the mass prophylaxis of protein deficiency. It looks as if protein-rich vegetables have to be the foods used—at least for the present, and investigators all over the world are seeking to devise a sufficiently good mixed vegetable protein diet for the prevention of protein malnutrition.

Some of the factors which have to be considered before such a mixture can be claimed as satisfactory are as follows:

(1) *Amino Acid Composition of the Protein:* The mixed protein should contain enough of each essential amino acid to supply the daily needs of the individual in the quantity in which it, along with the usual protein, is to be consumed. Further, the amino acids should show an adequately balanced pattern. It will be desirable to show these both chemically and biologically.

(2) *Toxicity:* Some vegetable proteins have been shown in the past (R. F. A. Dean, *Plant Proteins in Child Feeding*. M. R. C.

*Special Report Series No. 279 (1953)*) to contain various toxic principles which make them unsatisfactory to the human being. Untreated soy protein contains a trypsin inhibitor; peanut protein has a similar enzyme. For the protein to be accepted as safe, it must be shown to have no toxic principles and to develop none after processing or storage.

(3) *Storability in Tropical Climates:* This is perhaps one of the most difficult problems to be tackled. The tropical climate supports a wealth of insects and it is almost axiomatic that a highly satisfactory food for human beings will provide a good medium for other organisms. The fats and oils contained should withstand change so that the food does not lose its flavor. A food which requires refrigeration will be highly unsatisfactory; it will be impossible to transport over any great distance and the populations which need it most will not get it.

(4) *Ease of Preparation:* Most tropical housewives are overworked since they have to fetch the water and often mind the garden in addition to cooking for the family. Ideally then, any prophylactic food for a section of the family should be in a form which is ready to serve with the usual homecooked meals or requires at most some boiled soup for its final preparation.

(5) *Cost:* This is a factor which is often ignored in the experimental stages, but which in the long run is going to decide whether the food is a practical proposition or not. There are areas of Africa where a money economy does not exist. In other parts, whole families may have no more than a few cents a day to spend on "outside sources of food" and it would be too much to expect them to spend this little amount on food for say one toddler only. Perhaps in such really poor areas governments will have to

subsidize protein-rich foods. To keep costs down and for ease of acceptance, locally available ingredients need to be used if at all possible.

(6) *Acceptability and Availability to the Right Groups:* Many social, economic and educational factors influence the acceptance or rejection of a food by a community. A prophylactic food should accord, if possible, with the prevailing practice of the community.

If a food is aimed at the toddler group, some of the following factors must be taken into account before the final form of the food is determined:

(a) *Order at Meals:* Among most African households the one having first choice of any food is the husband or father. He is followed by the other "productive members" of the family, *i.e.*, the older sons and younger brothers. Other older children come next and often the mother with the toddlers are the last to be considered. Obviously a program aimed at the toddler will fail if the type of food used is acceptable to the others in the family, unless enough is available to satisfy the whole family.

(b) *The prestige value of the food:* The form a food takes may give it immense prestige or lack of prestige and such knowledge may be useful for bypassing the problem raised in (a) above. When skimmed milk was introduced into the old Gold Coast very little of it reached the toddler population for whom it was intended. It was either sold to get money for food "which children should eat", or it stood in paternal cupboards. Dean (*personal communication*) appears to have had success with "vegetable biscuits", but it is doubtful whether such a form of toddler food will be successful in rural Ghana. Biscuits have much prestige and they will almost certainly be handed over to the men folk. Foods which appear as gruel, on the other hand, have the reputation of being childrens' food and they are sure to be given to the children.

In this respect one has to strike a balance.

A food should not be so desirable as to be taken away from the toddler, and yet must be sufficiently acceptable for the mother to want to use it for the child. The prestige value of a food depends partly on its contents, partly on its appearance, and to some extent on local beliefs which may have little to do with either of these.

(c) *Taboos and Beliefs:* Although these have been mentioned from time to time, there has never been an effort to stress their importance in nutrition programs. In parts of Northern Ghana eggs are forbidden to women and first sons. In other areas pregnant women may not eat eggs, poultry or groundnuts and the list can be multiplied. Such knowledge requires that any prophylactic food having the above constituents may need to be well disguised or else accompanied by a large-scale educational campaign.

Most of these taboos appear odd to educated senses, but in some areas they are adhered to with feeling. Their origins are lost but, in the main, taboos like those mentioned above would appear to make protein-rich foods a predominantly male diet. The importance of such a situation becomes more apparent when it is realized that in particular areas poultry and groundnuts are the only protein-rich foods available.

(7) *Education of Government and Medical Personnel:* Doctors and paramedical personnel often need educating as to the problems at hand and the food being introduced. They must believe in it and be able to bear convincing witness to its efficiency. The education of governments has been neglected in the past and some fine programs have failed because of this (rice supplementation in the Philippines was stopped by government decree). Most of the developing areas of the world have strong central governments with leaders who are eager to achieve sound health for their people. The opportunity should be taken to educate such leaders as to the nutritional program at hand and, with

their support, a successful outcome is much more likely.

(8) *Educating the Population:* Once a new food is proved effective, safe and economical, a campaign should be launched to get the population to accept it and use it. People often change their foods and food habits and statements about resistance to change are not true in their entirety. A perfectly low-grade food like cassava has replaced corn in large areas of tropical Africa in recent years. This is due to the fact that cassava requires little effort for a good crop. It does not have to be stored since it can be left in the field until needed. This should serve as a lesson to nutritionists in their attempts to introduce new foods.

Apart from conformity to local dietary ideas and customs, the foods should require less financial or energy output than previous ones, or at any rate no more. They should be easy to handle, prepare and store, and their first introduction should not be attended by any mishap. In this respect, it is always worthwhile to have a food which

supplies other nutrients in sufficient quantities in addition to the protein being supplied. This would guard against the possibility of correcting one deficiency only to light up another. An unfortunate experience with a new food could set a nutrition and associated health program back a decade or more.

Nutrition has a real role to play in health and happiness, and, indirectly, in the actual rate of advance in the technically underdeveloped and developing areas. Protein malnutrition is far and away the most pressing nutritional problem in these areas today and it is at once a challenge and an opportunity for nutritionists, medical men and workers in all the other disciplines involved to try and do something about it. Action, however, should be based on careful study and a consideration of all the possible factors that would bear upon the success or failure of a program. Activism should not be a substitute for wise action.

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